Biochemical Pharmacology, Vol. 33, No. 15, pp. 2439–2445, 1984.
Printed in Great Britain.

0006-2952/84 $3.00 + 0.00
Pergamon Press Ltd.

IDENTIFICATION AND CHARACTERISATION OF L-[^{3}H] ASPARTATE BINDING SITES ON RAT SPINAL CORD SYNAPTIC MEMBRANES

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(*Received* 3 *January* 1984; *accepted* 22 *February* 1984)

Abstract—The binding of L-[^{3}H] aspartate to extensively-washed rat spinal cord synaptic membranes was investigated. Specific binding was enriched in synaptic membranes and was optimal under physiological conditions of temperature and pH. Equilibrium binding was established relatively slowly over a period of 30 min, and was totally reversible within 40 min. Saturation analysis revealed complex binding patterns. Two sites were clearly demonstrable, only one of which was shown to be saturable over the ligand concentration range employed in the study (0.1–10 μM). There was also some indication of the presence of a higher affinity site, although this was not investigated in any detail. Saturable binding demonstrated a K_D = 1.4 μM and B_{max} = 105 pmole/mg protein. Structure–activity studies with a range of amino acid analogues indicated that binding was stereospecific and was inhibited by a very restricted range of compounds. The most potent inhibitors of binding were L-glutamate and L-aspartate. There was no evidence for the involvement of NMDA receptors. Effects of possible endogenous modulators, including ions and guanosine nucleotides were investigated, and the chemical nature of the binding site probed with a number of protein-modifying agents.

A number of acidic amino acids such as L-glutamate and L-aspartate may function as excitatory transmitters in the mammalian spinal cord, although the evidence is less convincing than that for several areas of the brain. Glutamate and aspartate exhibit a heterogeneous distribution in the cord [1], and under hypoxic conditions which result in a selective loss of small spinal interneurones, a decrease in aspartate content has been reported [2]. More direct evidence for the possible role of aspartate has derived from electrophysiological studies: populations of spinal neurones possess a differential sensitivity to exogenously applied excitatory amino acids [3, 4] and these findings led to the suggestion that some spinal interneurones utilise L-aspartate as their excitatory synaptic transmitter.

Many neuropharmacological experiments directed towards the elucidation of the receptors involved in excitatory amino acid receptor function have been carried out in the feline [3–6] or rat [7] spinal cord. On the basis of their selective activation by the analogues, kainate, quisqualate and *N*-methyl-D-aspartate (NMDA), three classes of receptor have been proposed [8, 9]. The naturally occurring amino acids L-glutamate and L-aspartate may be considered as mixed agonists interacting with each of these three types, but probably showing some preference for one or other classes. For example, glutamate may be more quisqualate-like, while aspartate exhibits a preference for those receptors activated by NMDA [8, 9]. Within the last few years, a number of relatively selective antagonists have been developed, particularly some which are able to antagonise the actions of NMDA [8, 9]. In the spinal cord, these agents have been demonstrated to be effective inhibitors of polysynaptically-, and NMDA-evoked responses [5, 6].

Biochemical approaches to the characterisation of excitatory amino acid receptor types, have focussed largely on binding studies [10]. Binding sites for L-[^{3}H] aspartate have been described in the cerebellum [11], whole brain [12], and retina [13, 14]. However, it is striking that it was only in the latter tissue that NMDA was able to inhibit the binding of L-[^{3}H] aspartate. Attempts to label the NMDA site more specifically by using labeled NMDA have not been successful [15], although the labeled NMDA antagonists, 2-amino-5-phosphonovalerate (APV) and 2-amino-7-phosphonoheptanoate (APH) may be more useful in this respect [16, 17].

The object of this present study was to identify and characterise the binding sites for L-[^{3}H] aspartate on rat spinal cord synaptic membranes, in order that it might aid comparison of biochemical and electrophysiological data pertaining to excitatory amino acid receptor types, in the same preparation.

MATERIALS AND METHODS

(a) *Synaptic membranes*. Albino Wistar rats (250–300 g), either sex) were killed by decapitation and the spinal cords removed rapidly. These were then homogenised in 20 vol of 0.32 M sucrose buffered with 5 mM Hepes–KOH buffer (pH 7.4). After homogenisation with a Teflon–glass homogeniser (0.1 mm clearance, 5 strokes at approximately 1000 rpm) the homogenate was centrifuged at 1000 *g* for 10 min in a J2-21 centrifuge. The supernatant was collected and centrifuged at 17,000 *g* for 20 min to obtain the P2 pellet. After resuspension in 0.32 M

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sucrose/5 mM Hepes–KOH buffer (pH 7.4), approximately 4 ml aliquots were layered onto a two-step sucrose gradient consisting of 0.8 and 1.2 M sucrose, each buffered with 5 mM Hepes–KOH (pH 7.4). These were centrifuged in a Beckman L5.65 ultracentrifuge using an SW-27 swing-out rotor at 100,000 *g* for 60 min. The myelin fraction (above the 0.8 M sucrose layer) or the mitochondrial pellet, were occasionally collected. Synaptosomes were harvested from the 0.8/1.2 M sucrose interface and after the addition of excess 5 mM buffer, were re-centrifuged at 50,000 *g* for 20 min. The pellet was then thoroughly lysed/disrupted in fresh 5 mM Hepes–KOH buffer (pH 7.4) using a tight-fitting glass–glass homogeniser, and allowed to stand on ice for 10 min. This was followed by centrifugation at 50,000 *g* for 20 min. This washing procedure was repeated twice by resuspension in fresh buffer with subsequent centrifugation. The synaptic membrane-enriched pellet was resuspended finally in 10 vol (original wet weight) of 50 mM Hepes–KOH buffer (pH 7.4).

(b) L-[^{3}H] *aspartate binding assay*. Assays were carried out in 1.9 ml polypropylene microcentrifuge tubes (Elkay Inc., MA., U.S.A.). To each tube was added 25 μl of L-[^{3}H] aspartate (5.1 or 9.6 Ci/mmole specific radioactivity. Amersham International) and either 25 μl buffer (total binding) or 25 μl 1mM L-aspartate to define non-specific binding. In experiments using a fixed ligand concentration, this was 60 nM (30 nM label, diluted with 30 nM unlabeled L-aspartate). Binding assays were initiated by the addition of 500 μl of the membrane suspension and were continued for 10 or 30 min at 37° in a shaking water bath. Assays were terminated by centrifugation for 30 sec in a Beckman microfuge B, which effectively pelleted all the membrane constituents. The supernatant was immediately aspirated, and the pellet superficially washed with care, using 1 ml of buffer, followed by its immediate aspiration. The microfuge tube tips were cut off and transferred to scintillation vials, where the tissue pellets were solubilised overnight in 2% SDS. After the addition of scintillant (xylene 5.34 ml synperonic NXP, 2.66 ml PPO, 32 mg and dimethylPOPOP, 4 mg) bound radioactivity was measured in a Beckman LS7500 liquid scintillation spectrometer. Specific binding was determined by subtraction of the non-specific from the total binding component.

(c) *Materials*. All common analytical reagents and drugs were obtained from either Sigma (UK) Ltd., or B.D.H. Chemicals. Ibotenate was a gift of Professor C. H. Eugster (Zurich), and phosphonate analogues of excitatory amino acids were from J. F. Collins (London) and J. C. Watkins (Bristol). DGG and cis PDA were from J. C. Watkins, and other analogues were purchased from Cambridge Research Biochemicals.

RESULTS

(a) *Effects of temperature and pH on specific* L-[^{3}H] *aspartate binding*. Under the normal assay conditions, the specific binding of L-[^{3}H] aspartate usually represented 70–80% of the total binding. Binding was found to be optimal at pH 7.4 and at a

Table 1. Effect of pH and temperature on specific L-[^{3}H]-aspartate binding to spinal cord synaptic membranes

	Specific binding (pmole/mg · protein)
(a) pH	
6	0.402 ± 0.042
7	0.606 ± 0.036
7.4	1.024 ± 0.098
8	0.517 ± 0.060
9	0.127 ± 0.054
(b) Temperature (°)	
0	0.244 ± 0.10
22	0.74 ± 0.122
37	0.956 ± 0.177
50	0.020 ± 0.084

Freshly prepared spinal cord synaptic membranes were incubated for 10 min with 60 nM L-[^{3}H] aspartate in the absence, or presence, of L-aspartate (1 mM). Assay conditions were as described in the text. Results are the mean ± S.E.M. from duplicated experiments performed in quadruplicate.

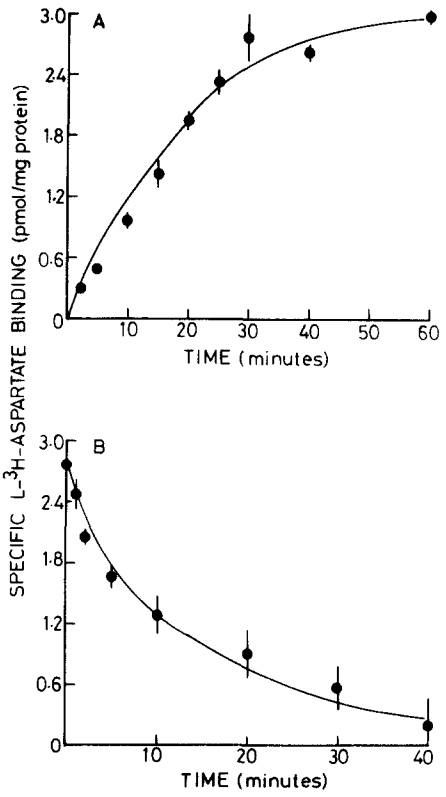

Fig. 1. (A) Association of L-[^{3}H]-aspartate with spinal cord synaptic membranes. Incubations were performed at 37° in 50 mM Hepes–KOH buffer (pH 7.4) and 60 nM L-[^{3}H]-aspartate. Specific binding was defined by addition of 1 mM L-aspartate. Results are the mean ± S.E.M. of quadruplicate determinations from three independent experiments. (B) Dissociation of L-[^{3}H]-aspartate (60 nM) from spinal cord synaptic membranes was studied following a 30 minute incubation at 37° by addition of a large excess of unlabeled L-aspartate (1 mM) to the assay.

Table 2. Subcellular distribution of specific L-[^{3}H]-aspartate binding

Tissue fraction	Specific binding (pmole/mg protein)
P_1 supernatant	0.186 ± 0.052
P_2 supernatant	0.034 ± 0.020
P_2 pellet	0.331 ± 0.066
Crude myelin	0.173 ± 0.163
Mitochondrial	0.269 ± 0.105
Synaptosomal	0.383 ± 0.018
Washed synaptic membranes	0.791 ± 0.089

Spinal cords were fractionated as described in the text. The specific binding of L-[^{3}H]-aspartate (60 nM) was defined as that displaced by added 1 mM unlabeled L-aspartate. Incubations were performed at 37° for 10 min. Results are the means ± S.E.M. of quadruplicate determinations.

temperature close to 37° (Table 1a and b). These conditions correspond to those likely to be encountered in the vicinity of the receptor *in vivo*. Specific binding was much more sensitive to changes in either the pH of the assay medium, or incubation temperature than was the non-specific component (data not shown). Binding was linear with protein concentration in the range used in these experiments (100–200 μg/assay tube). This indicates that neither receptor–ligand dissociation nor protein degradation occurs following termination of the assay (data not shown).

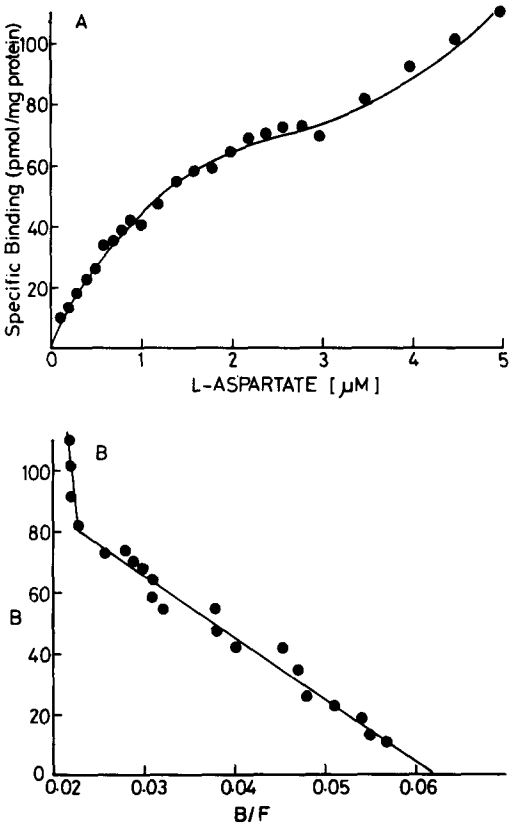

Fig. 2. Saturation of L-[^{3}H]-aspartate binding to rat spinal cord synaptic membranes investigated over ligand concentration range of 0.1–10 μM. Synaptic membranes were incubated at 37° for 30 min and specific binding was defined as that displaced by addition of L-aspartate (1 mM). The lower plot shows the data presented as an Eadie–Hofstee plot. Kinetic parameters were calculated as described in the text. Results are the mean ± S.E.M. of three independent experiments performed in quadruplicate.

(b) *Time course and reversibility of specific* L-[^{3}H] *aspartate binding.* Specific binding of L-[^{3}H] aspartate was found to increase slowly and to attain equilibrium after approximately 30 min (Fig. 1A). Half-maximal binding was seen after 12 min. The non-specific binding was essentially instantaneous and did not increase, even after prolonged incubation (not shown). Addition of a large excess of unlabeled aspartate (1 mM) demonstrated that specific binding was totally reversible within 40 min (Fig. 1B). A typical exponential decay curve was found, with 50% of the specific binding being displaced after approximately 10 min. No evidence was found for either multi-component association or dissociation processes.

(c) *Subcellular distribution of specific* L-[^{3}H] *aspartate binding.* Specific binding of L-[^{3}H] aspartate was found to be enriched in fractions containing synaptic membranes (Table 2). Following sucrose density gradient centrifugation, enhanced binding was found in synaptosomal, rather than in the myelin or mitochondrial fractions. Binding to these latter fractions probably represents synaptosomal contamination. Binding was increased by a further 200% following lysis and extensive washing of synaptosomes. The overall enrichment in specific binding was 4.3-fold.

(d) *Stability of* L-[^{3}H] *aspartate binding sites.* The effect of freezing the tissue on specific L-[^{3}H] aspartate binding was investigated. Freezing of the final synaptic membrane suspension at −4° for 24 hr produced a 50% decrease in binding. If intact tissue was frozen, either in liquid nitrogen, or in liquid nitrogen-cooled 5 mM Hepes–KOH buffer (pH 7.4), specific binding could be retained at control levels (data not shown). However, these conditions did result in a significant decrease in specific binding as a percentage of the total detected. Since this basic characteristic of binding was thus altered, frozen membranes were not employed in any further experiments. Lyophilisation of membranes led to a slight increase in binding, as compared with control levels. The equilibrium control binding level of 2.7 pmole/mg protein (established by 30 min) was maintained for incubations of up to 90 min.

(e) *Saturability of* L-[^{3}H] *aspartate binding.* The specific binding of L-[^{3}H] aspartate was determined under equilibrium conditions, using a wide range of ligand concentrations (50 nM–5 μM). A complex pattern of binding was observed, with two sites being defined clearly. One site was saturable, while the other was not, within the defined concentration limits (Fig. 2). When binding was investigated further within the 1–50 nM concentration range, there was some indication that other higher affinity sites were present (data not shown). The non-specific binding of L-[^{3}H] aspartate was linearly related to the ligand concentration over the entire range investigated. Kinetic characterisation of saturable binding was

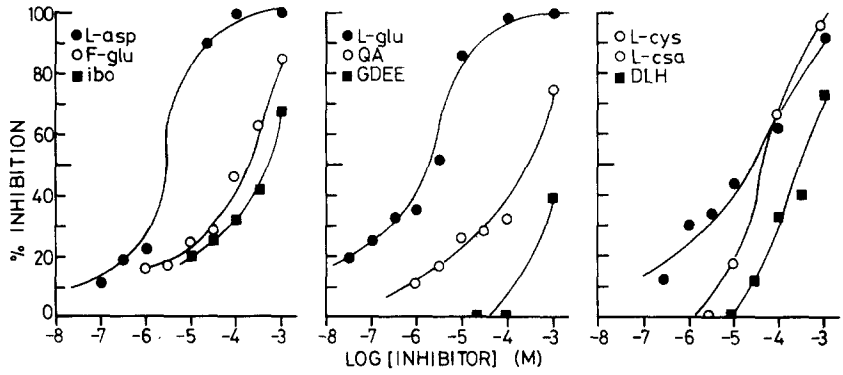

Fig. 3. Inhibition of L-[^{3}H]aspartate binding to rat spinal cord synaptic membranes by excitatory amino acid analogues. Synaptic membranes were incubated for 30 min at 37° with 60 nM L-[^{3}H]aspartate in the presence, or absence of amino acid derivatives. Results are the mean ± S.E.M. of quadruplicate determinations from usually three independent experiments. IC_{50} values were read directly from these plots. Abbreviations: F-glu, DL-fluoroglutamate; ibo, (±)-ibotenate; QA, quisqualate; GDEE, L-glutamate diethylester; L-cys, L-cysteate; L-CSA, L-cysteine sulphinate; DLH, DL-homocysteate.

performed using either a computer-derived curve fitting programme [18] or by Eadie–Hofstee analysis [19]. The dissociation constant, K_D was found to be 1.40 ± 0.39 μM, and the binding site density, B_{max} was 104.6 ± 13.98 pmole/mg protein. Approximate values were also obtained for the site which did not saturate under our experimental conditions: the K_D was approximately 16 μM and the B_{max} 415 pmole/mg protein.

A Hill plot was constructed using data from the saturable binding component, and this revealed a slope of 1.05 ± 0.02, indicating lack of cooperativity or site heterogeneity.

Table 3. Inhibition of L-[^{3}H]-aspartate binding to rat spinal cord synaptic membranes by excitatory amino acid analogues

Compound	IC_{50} (μM)	Hill slope
L-Glutamate	1.85	0.9
L-Aspartate	2.71	1.0
L-Cysteate	27.1	0.96
L-Cysteine sulphinate	46.6	0.9
4-Fluoroglutamate	158	0.93
DL-Homocysteate	251	0.92
(±) Quisqualate	271	0.96
(±) Ibotenate	631	0.95
GDEE	1359	0.98

$IC_{50} \simeq 1$ mM: (±)APP, (±)APB, D-aspartate, D-glutamate threo-3-hydroxyaspartate, DL-α-aminoadipate, DL-α-aminosuberate, DL-α-ε-diamino-propionate.

Not effective: cPDA, NMDA, quinolinate, (±)APV, (±)APHx, (±)APH, kainate, γ-DGG, HA966, phencyclidine, carbachol, noradrenaline, GABA, glycine, sodium pentobarbitone.

Synaptic membranes were incubated at 37° for 30 min with 60 nM L-[^{3}H]-aspartate in the absence or presence of a wide range of concentrations (10 nM–1 mM) of the compound under test. IC_{50}s (the concentration required to produce 50% inhibition for that compound) were read directly from log concentration percentage inhibition curves (see Fig. 3). Hill slopes were determined from appropriate plots of these data.

(f) *Inhibition of specific* L-[^{3}H] *aspartate binding.* A number of compounds were tested over a wide concentration range (10 nM–1 mM) for their ability to inhibit the binding of L-[^{3}H] aspartate. IC_{50} values were determined from log concentration/percentage inhibition curves (Fig. 3) using a ligand concentration of 60 nM (Table 3). A restricted range of compounds was found to inhibit binding. The most potent substances were L-aspartate and L-glutamate, which were approximately equipotent. The D-isomers of these substances were far less potent, indicating that the site is stereoselective. Less effective inhibitors of binding were the L-isomers of cysteate and cysteine sulphinate, as well as fluoroglutamate. Ibotenate, quisqualate and DL-homocysteate were all active, although they were less potent than the other active agonists. All of the inhibition curves for these compounds exhibited Hill slopes of close to unity. A number of other compounds were found to possess weak inhibitory activity on L-[^{3}H] aspartate binding, namely: L-glutamate diethylester (GDEE), DL-2-amino-3-phosphonopropionate (APP), DL-2-amino-4-phosphonobutyrate (APB), DL-alpha-aminosuberate, DL-α,ε-diaminopropionate and cis-piperidine-2,3-dicarboxylate (cisPDA). NMDA, kainate, quinolinate, gamma-D-glutamyglycine, DL-2-amino-

Table 4. Effect on ions on specific L-[^{3}H]-aspartate binding

Ion added	Specific binding (pmole/mg protein)
Control	1.05 ± 0.014
100 mM NaCl	2.39 ± 0.172
5 mM KCl	0.79 ± 0.033
2.5 mM CaCl	1.31 ± 0.055
100 mM NH_4Cl	1.05 ± 0.031
100 mM ammonium acetate	1.10 ± 0.020

Spinal cord synaptic membranes were incubated in 50 mM Hepes–KOH buffer (pH 7.4) for 10 min with L-[^{3}H]-aspartate (60 nM) in the absence or presence of ions. Specific binding was determined as described in the text. Results are mean ± S.E.M. from at least two independent experiments performed in quadruplicate.

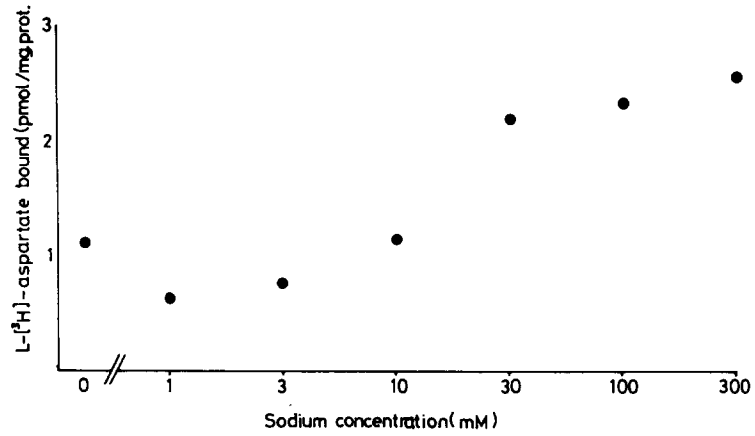

Fig. 4. Effect of varying sodium concentration (1–300 nM) on the specific binding of L-[^{3}H]-aspartate (60 nM). Synaptic membranes were incubated at 37° for 10 min and specific binding was defined by addition of L-aspartate (1 mM). Results are the mean ± S.E.M. of two independent experiments performed in quadruplicate.

5-phosphonovalerate (APV), DL-2-amino-6-phosphonohexanoate (APHx) and DL-2-amino-7-phosphonoheptanoate (APH) were inactive, as were compounds unrelated to excitatory amino acids, such as noradrenaline, carbachol, GABA, glycine and pentobarbitone.

(g) *Effect of ions on specific* L-[^{3}H] *aspartate binding.* The specific binding of L-[^{3}H] aspartate was determined in the presence of various added ions at their presumed physiological concentrations (Table 4). Binding of 60 nM labeled aspartate in the absence of ions was found to be 1.05 ± 0.014 pmole/mg protein. Sodium ions (100 mM) increased binding to 2.39 ± 0.17 pmole/mg protein, while calcium ions produced a smaller increase in binding to 1.31 ± 0.05 pmole/mg protein. Potassium ions (5 mM) inhibited binding by approximately 25% to a level of 0.79 ± 0.03 pmole/mg protein. Each of these ions was added as the chloride salt. Addition of ammonium ions, either as the chloride or acetate salts, did not influence binding. This clearly suggests that the effects of these ions are not attributable to non-specific ionic strength effects.

Further investigation of the effect of sodium ions revealed that this response was concentration dependent (Fig. 4). Low concentrations of sodium ions (<10 mM) inhibited binding by around 40%. The enhancing effect of sodium was only seen at higher concentrations (>10 mM) and is likely to be due to recruitment of sodium-dependent uptake binding sites.

(h) *Effect of protein modification on specific* L-[^{3}H] *aspartate binding.* A number of protein-modifying agents were used in order to investigate the chemical nature of the L-[^{3}H] aspartate binding site (Table 5). Compounds that altered the oxidation state of sulphydryl groups, such as DTT and DTNB, did not influence binding. Alkylation of sulphydryl groups using NEM, or carboxyl group modification using carbodiimide, produced large significant increases in binding. Other treatments such as the exposure of synaptic membranes to azide to achieve chelation of protein-associated metal ions, or interference with glycoprotein residues with concanavalin A, produced small, but statistically non-significant changes in binding. Protease inhibitors such as PMSF/EDTA did not influence binding.

Table 5. Effect of protein modifying agents on specific L-[^{3}H]-aspartate binding

Addition	Specific binding (pmole/mg protein)
—	1.20 ± 0.20
3 mM NEM	2.02 ± 0.12
3 mM DTT	1.19 ± 0.09
10 mM DTNB	1.16 ± 0.19
20 mM carbodiimide	2.14 ± 0.49
200 μg/ml concanavalin A	1.34 ± 0.39
0.33 mM Sodium azide	0.880 ± 0.389
0.1 mM PMSF/0.5 mM EDTA	1.02 ± 0.18

Spinal cord synaptic membranes were incubated in 50 mM Hepes–KOH (pH 7.4) buffer in the presence or absence of the above protein modifiers for 10 min at 37° with L-[^{3}H]-aspartate (60 nM). Results are from quadruplicate experiments. Abbreviations: NEM, *N*-ethyl-maleimide, DTT, dithiothreitol, DTNB, 5,5′-dithiobis (2-nitrobenzoic acid). PMSF, phenylmethylsulphonyl fluoride; the carbodiimide used was l-ethyl-3(3-dimethylamino propyl) carbodiimide.

DISCUSSION

In this study, we have demonstrated the presence on spinal cord synaptic membranes, of binding sites for L-[^{3}H] aspartate. These may represent the receptor sites that are occupied *in vivo* by this ligand, although further exhaustive studies are necessary before this may be assumed with any certainty. It seems unlikely that binding to sodium-dependent uptake sites makes a major contribution to the binding observed under our experimental conditions, since the potent uptake inhibitor threo-3-hydroxyaspartate, was devoid of significant inhibiting effects on aspartate binding. The fact that the binding of

aspartate was optimal at physiological pH and temperature, and that it deteriorated markedly with extremes of these conditions, suggests the involvement of a protein molecule in binding. Furthermore, the enrichment of binding in synaptic elements is wholly consistent with an interaction with a receptor molecule. The significant increase in binding following lysis and extensive washing of the membranes is likely to be due to the removal of endogenous transmitters (glutamate, aspartate etc.) and possibly other endogenous inhibitors [10].

A number of other studies have utilised labeled aspartate as a potential receptor probe [11–14], although comparison between findings is complicated by differences in membrane preparation and in regions of nervous tissue employed. The time course of binding in the spinal cord, was similar to that found in whole brain membranes [12], although somewhat slower than that for cerebellum [11]. Saturation analysis revealed a complex picture quite dissimilar from those found in previous studies [11, 12]. A saturable site was demonstrated, with a K_D in the low micromolar range, which was similar to that found in whole brain and cerebellum. However, we also detected what appeared to be a site of much higher affinity (K_D in the nanomolar range) and an additional one of such low affinity that it did not saturate under the conditions employed in these studies. It is possible that this binding site is associated with a specific enzyme or transport site. However, further investigation is required. An additional possibility to be considered, is that each of the binding site types might be conformationally-linked forms of the same receptor protein. However, Hill plots revealed slopes close to unity in all cases, and thus would argue against this possibility.

The possible role of endogenous regulators of binding was investigated, paying particular attention to the involvement of certain ions. In common with its effects on a number of receptor systems, including L-glutamate [20], low concentrations (i.e. below those likely to be experienced in the receptor environment *in vivo*) of sodium resulted in an inhibition of binding. Although this may mean that sodium has no physiological regulatory role, this possibility cannot be discounted. Higher concentrations, i.e. > 10 mM, enhanced binding substantially, and probably represents at least in part, the recruitment of uptake sites. The effects seen with both calcium ions (enhancement) and potassium (small inhibition of binding) occurred at concentrations likely to be encountered in the synaptic cleft. A similar pattern of ionic regulation was found to occur in the cerebellum [11]. In contrast to the binding of L-[^{3}H] glutamate or DL-[^{3}H] APB [21], no evidence was found to suggest that in the spinal cord chloride ions were able to enhance specific binding of L-[^{3}H] aspartate.

Other possible regulators of binding that have been reported to influence the interaction of a number of transmitters with their (usually cyclase-linked) receptors, include the guanosine nucleotides [22, 23]. It was therefore of interest to find that in this study, the binding of L-[^{3}H] aspartate was also inhibited to some degree (approx 30%) (data not shown) by Gpp(NH)p and GTP in particular. The complex binding patterns observed with aspartate precluded assessment of the mechanisms of action of any of these regulatory substances.

In order to gain some insight into the chemical nature of the sites labeled by L-[^{3}H] aspartate, we investigated the effects of a number of well established protein modifying agents on specific binding. While the data revealed that irreversible modification of sulphydryl or hydroxyl groups strongly enhanced binding, the use of other probes designed to identify the involvement of metalloproteins or glycoproteins did not permit firm conclusions to be drawn. Since protease inhibitors such as PMSF/EDTA were unable to influence binding, peptide fragments are unlikely to be involved in the binding process. Attempts were made to expose selectively the high affinity L-[^{3}H] aspartate binding site using protein modifiers or ions, but this proved unsuccessful.

Receptor sites for NMDA have been suggested to exhibit a clear preference for aspartate amongst the endogenous excitatory amino acid transmitter candidates [4]. Since the electrophysiological/neuropharmacological characterisation of the NMDA site has been pursued extensively in the mammalian spinal cord [8, 9], it was anticipated that binding studies carried out with L-[^{3}H] aspartate in this tissue might reveal a distinct population of NMDA-sensitive sites. In fact, investigation of the structure–activity profile of binding revealed that the major binding site interacted with only a very limited number of acidic amino acid derivatives. Binding was stereospecific, with the L isomers of aspartate and glutamate being the most effective inhibitors of binding. The sulphur-containing amino acids were also active, and these substances may show a preference for the quisqualate type of receptor. While ibotenate, which exhibits activity at NMDA (but also at other receptor classes) inhibited binding, albeit rather weakly, NMDA itself and a range of potent phosphonate inhibitors, which act selectively at NMDA receptors, failed to modify binding. Previous studies in the cerebellum [11] and whole rat brain [12] also did not show labelling of what might be considered as NMDA sites. Indeed, the pharmacological specificity of binding yielded no clear cut profile that was interpretable in terms of the electrophysiologically-defined 3 receptor site model [8]. The pharmacological characteristics of binding do differ between several of the studies [11–14] and, in the retina at least, NMDA sensitivity was demonstrated. However, the characteristics of this site differed in practically all other respects from our own, and those of the other studies. What therefore can be concluded about the binding site characteristics for L-[^{3}H] aspartate on spinal cord membranes? In a number of respects, binding is compatible with interaction with a glutamate/quisqualate-preferring site. However, a number of anomalies were evident, such as the marked inactivity of AMPA and APB at this site, thus firm conclusions cannot be drawn at this stage.

Acknowledgements—This work was supported by a project grant from the Science and Engineering Research Council to P. J. R. We thank Lynn Wilkinson and Gill Jackson for secretarial assistance.

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