Structural specificity of high affinity [^{3}H]-glutamate uptake in striatal homogenates

STEVEN R. VINCENT, EDITH G. MCGEER*, *Kinsmen Laboratory of Neurological Sciences, Department of Psychiatry, University of British Columbia, Vancouver, B.C., Canada V6T 1W5*

A high affinity uptake system for the excitant amino acids has been demonstrated in neural tissue (Logan & Snyder 1971). This uptake process is thought to be the major mechanism by which synaptically released amino acids are inactivated. Drugs which interfere with this process can potentiate the excitant actions of the acidic amino acids (Curtis et al 1970). Also, it is known that high concentrations of glutamic acid can kill neurons (Olney 1969; McGeer & McGeer 1976), and thus the high affinity uptake system may be important in maintaining glutamate concentrations below toxic concentrations.

Recently, evidence from electrophysiological (Spencer 1976) and biochemical studies (Divac et al 1977; McGeer et al 1977; Kim et al 1977) have indicated that the striatum is innervated by a glutamate system arising in the cerebral cortex. A population of receptors for the glutamate analogue [^{3}H]kainic acid has been demonstrated on striatal neurons (Vincent & McGeer 1979), and a consistent decrease in glutamate levels (Kim et al 1977) and in the high affinity uptake of glutamate has been observed in the striatum following lesions of the cortical afferents (Divac et al 1977; McGeer et al 1977). Thus the striatum provides a good system in which to examine the structural specificity of high affinity glutamate uptake in the central nervous system.

Male Wistar rats (300 g) were killed by cervical fracture, the striata dissected on ice, and homogenized in 50 volumes of 0·32 M sucrose in 5 mM sodium phosphate buffer, pH 7·4 in a glass-Teflon homogenizer.

* Correspondence.

Table 1. Inhibition of the high affinity uptake of L-glutamate into rat striatal homogenates. Striatal homogenates were assayed with 500 μM of inhibitor in the presence of 1 μM[^{3}H]glutamate. Results are the mean ± s.e.m. for triplicate determinations. Inhibition produced by 500 μM L-glutamate defined as 100%.

Inhibitor (500 μM)	% inhibition of uptake
L-Cysteine sulfinic acid	100
L-Glutamate	100 ± 7
L-Aspartate	99 ± 7
2-Amino-3-phosphonopropionic acid	86 ± 9
Kainic acid	84 ± 8
N-Methyl aspartic acid	79 ± 6
D-Glutamate	54 ± 6
γ-Aminobutyric acid	36 ± 4
Glutamic acid diethyl ester	36 ± 5
L-Glutamine	27 ± 5
DL-Homocysteic acid	23 ± 4
2-Amino-4-phosphonobutyric acid	23 ± 3
α-Amino adipic acid	20 ± 5
Ibotenic acid	18 ± 3

High affinity uptake of glutamate was measured on this homogenate according to the method described by Divac et al (1977) at a final glutamate concentration of 1 μM. All assays were run in triplicate, and identical samples incubated at 4 °C served as blanks. Potential inhibitors of glutamate uptake were pre-incubated at 500 μM final concentration for 5 min with the striatal homogenate at 25 °C before addition of radioactive substrate. Incubation was continued for 3 min followed by dilution with 4 ml ice-cold 0·9% NaCl and filtration onto 0·45 μM Millipore filters.

The results of the inhibition studies are presented in Table 1. In agreement with earlier studies on rat brain slices (Balcar & Johnston 1972), L-cysteinesulfinate, L-glutamate and L-aspartate were equally effective inhibitors of L-[^{3}H]glutamate uptake. Also in agreement with this previous report is the observed lack of significant effect of ibotenate, L-glutamate diethyl ester, L-glutamine, GABA, and α-amino adipic acid on glutamate uptake.

In agreement with a previous report from this laboratory (McGeer et al 1978) we have observed a significant inhibition of high affinity glutamate uptake by the potent neuroexcitant kainic acid. *N*-Methyl aspartate, another potent neuroexcitant was also effective in inhibiting glutamate uptake. These observations agree with the results which Lakshmanan & Padmanaban (1974) obtained with synaptosomes. Also, Johnston et al (1979) have reported kainic acid to be a linear competitive inhibitor of high affinity glutamate uptake, although these workers found *N*-methyl asparatate to be without effect on rat brain slices.

While the aspartate-like drug 2-amino-3-phosphonopropionic acid is a potent inhibitor of glutamate uptake, the glutamate-like analogue 2-amino-4-phosphonobutyric acid was without effect. Also, D-glutamate is a poor inhibitor, while D-aspartate is reported to be a more powerful inhibitor than the corresponding L-isomer (Balcar & Johnston 1972). This, together with the remarkable potency of *N*-methyl aspartate suggests that although both L-glutamate and L-aspartate are substrates of comparable affinity for the high affinity uptake system, the uptake site prefers aspartate-like compounds, and may have characteristics similar to the aspartate receptor. This work was supported by grants from the Medical Research Council of Canada and the Huntington's Chorea Foundation. S.R.V. is an MRC student.

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Mianserin, danitracen and amitriptyline withdrawal increases the behavioural responses of rats to L-5-HTP

E. MOGILNICKA*, V. KLIMEK, *Institute of Pharmacology, Polish Academy of Sciences, 12, Smetna-Str., 31-343 Kraków, Poland*

Trulson et al (1976a,b) demonstrated that after lesion of the central 5-hydroxytryptamine (5-HT) nerve terminals in rats supersensitivity to 5-HT precursors and agonists occurred. Such supersensitivity was not observed after a chronic inhibition of the 5-HT synthesis or a chronic blockade of 5-HT receptors by methysergide (Trulson et al 1977). On the other hand, Klawans et al (1975, 1977) found an enhanced behavioural response to D,L-5-hydroxytryptaphan after a chronic treatment of guinea-pigs with methysergide.

The present study was undertaken to determine whether a repeated administration of mianserin and danitracens, both antidepressant drugs that block 5-HT receptors (Kähling et al 1975; Maj et al 1976a, b; Van Riezen 1972), affected the response to 5-HTP. For comparison we also examined amitriptyline, the classical tricyclic antidepressant regarded as an inhibitor of the 5-HT and noradrenaline (NA) uptake. The 'wet-dog' shake behaviour reflecting the activity of the central 5-HT system (Bedard & Pycock 1977) was used to test the responsiveness to L-5-HTP.

Male Wistar rats (180–210 g) were placed individually in wire mesh cages and immediately after injection of L-5-HTP ethyl ester HCl (dissolved in 0·9% NaCl, injected i.v. in a constant volume of 1 ml kg^{-1}, 12·5 mg kg^{-1}, the dose producing about 20 shakes per 30 min) wet dog shakes were recorded for 30 min. Shakes began about 3 min after L-5-HTP injection, reached a peak about 10–15 min later and lasted for almost 45 min. Apart from the shakes, the rats occasionally displayed reciprocal forepaw treading (rhythmical dorsoventral movements of the forelimbs), forepaw crossing, grooming with the forepaws and hind limbs as well as sporadic yawning. The animals were treated with a vehicle or drugs injected once or twice a day at 9 a.m. and 6 p.m. for 4 or 10 days. The last drug dose (except for amitriptyline, the last dose of which was given 72 h before) was administered 24, 48 or 72 h before injection of L-5-HTP ethyl ester.

A single dose of mianserin (2 mg kg^{-1}) (Fig. 1A) 24, 48 and 72 h before L-5-HTP did not affect the number of L-5-HTP-induced shakes. A 4-day administration (Fig. 1B) markedly elevated the number of shakes 48 and 72 h after withdrawal. A 10-day treatment (Fig. 1C) significantly reduced the number of shakes when the last dose of mianserin was administered 48 h before L-5-HTP, and increased it markedly when mianserin was given 72 h before.

A single dose of danitracen (3 mg kg^{-1}) (Fig. 1A), 72 h before L-5-HTP, elevated (by about 50%) the number of shakes. A similar but much stronger effect was observed after 72 h with 4- and 10-day danitracen injections (Fig. 1B and 1C). In this 4- and 10-day treatment the number of shakes was reduced significantly after 24 h in the latter regimen. After 48 h the results did not differ from the control values.

A single dose of amitryptyline (10 mg kg^{-1}) (Fig. 1A) 72 h before the test had no effect on the L-5-HTP-induced shakes, whereas 4- and 10-day treatment significantly increased the number of shakes (Fig. 1B and 1C).

The results indicate that mianserin and danitracen in doses blocking 5-HT receptors, e.g. blocking the head shake response to L-5-HTP (Maj et al 1976a; 1978) enhance this response when they are administered chronically. Since the enhanced response to L-5-HTP occurs particularly 72 h after withdrawal of mianserin, danitracen, and not 24 h after, it may be assumed that it does not result from the effect of the examined drugs on pharmacokinetics or the L-5-HTP metabolism. It seems, therefore, that this response may be due to a prolonged (4- or 10-day) blockade of 5-HT receptors, as was observed with methysergide (Klawans et al 1975). The lack of an enhanced response to L-5-HTP in the initial period (especially after 24 h), or even its weakening (after a 10-day danitracen administration)

* Correspondence.