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Distinct pharmacological properties of excitatory amino acid receptors in the rat striatum: Study by Na^+ efflux assay

(glutamate analogs/agonists/antagonists/putative neurotransmitters/brain slices)

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ABSTRACT **Specific $^{22}Na^+$ efflux rates from preloaded rat striatal slices are increased in a dose-dependent manner by L-glutamate and other excitatory amino acids displaying the following order of efficiency: *N*-methyl-D-aspartate > DL-homocysteate > quisqualate > kainate > D-glutamate > L-glutamate > L-aspartate. Amino acid antagonists such as 2-amino-5-phosphonovalerate, γ-D-glutamylglycine, DL-aminosuberate, DL-aminoadipate, and diethyl glutamate but not nonexcitatory amino acids such as γ-aminobutyric acid inhibit the amino acid-induced increase in specific $^{22}Na^+$ efflux rate. Increased K^+ concentrations, in the presence of 2 mM Ca^{2+}, increase the specific $^{22}Na^+$ efflux. The latter and the response to *N*-methyl-D-aspartate, but not the responses to L-glutamate, L-aspartate, quisqualate, and kainate, are inhibited to similar extents by the same antagonists. These results suggest the release from striatal nerve terminals of a putative neurotransmitter with pharmacological properties different from those of L-glutamate or L-aspartate but similar to those of *N*-methyl-D-aspartate. The results of this study show that the stimulation of the $^{22}Na^+$ efflux in brain slices by neuroactive amino acids and K^+ ions is a valid and powerful tool for pharmacological investigations of excitatory amino acid receptors and their putative ligands.**

The excitatory actions of dicarboxylic amino acids in the mammalian central nervous system are mediated by their interactions with specific receptors in the neuronal membrane. The existence of these excitatory amino acid receptors has been inferred from observations of differential sensitivities of neurons to the excitatory effects of structural analogs of glutamate and aspartate (1–6) and recently, from the use of selective antagonists of amino acid-induced excitation (6–11).

The present state of knowledge about amino acid excitation in the central nervous system has been reached mainly from electrophysiological studies, in which the sensitivity of mammalian neurons to excitatory amino acids has been examined mainly *in vivo*, and also in brain slices (12, 13) and in neuronal cultures *in vitro* (14–16). So far, only a few attempts have been made to investigate the amino acid-induced excitation by alternative methods (17, 18).

Biochemical methods based on measurements of ion movements that result from neurotransmitter receptor interactions have been of great assistance in the study of the receptors of acetylcholine (19, 20), γ-aminobutyric acid (21), and norepinephrine (22).

In brain slices, ion movements induced by L-glutamate (L-Glu) and other excitatory amino acids were measured (17, 18), but these ion movements have not been exploited in pharmacological studies of amino acid receptors.

In this paper, we describe a biochemical method, based on measurements of ions effluxes from brain slices, that greatly facilitates the *in vitro* investigation of the pharmacological properties of functional amino acid receptors and of their natural ligands.

MATERIALS AND METHODS

Measurements of $^{22}Na^+$ Efflux. Male Charles River rats (45–55 days old) were killed by decapitation. The brain was removed and the two striata were isolated (23). Sections (300 μm thick) were prepared by using a McIlwain chopper and placed within 4 min after sacrifice into basket-shaped sieves (three to six slices per sieve) kept at 37°C in an oxygenated physiological medium (124 mM NaCl/0.76 mM KCl/1.24 mM KH_2PO_4/1.2 mM $MgSO_4$/2 mM $CaCl_2$/10 mM glucose/26 mM Tris·HCl adjusted to pH 7.3). After 30–40 min, the slices were transferred and incubated for 20 min in 0.7 ml of a physiological medium containing $^{22}Na^+$ (Radiochemical Centre, Amersham, England) at 2 μCi/ml (1 Ci = 3.7×10^{10} becquerels).

The wash-out of the radioactivity from the extracellular space and the exposure of the slices to the various effectors were carried out according to the following procedure: After the $^{22}Na^+$ loading period, the slices were transferred with the help of the sieve every minute through a series of 28 tubes, each containing 2 ml of a nonradioactive physiological solution saturated with O_2. The effectors to be tested were present in the last 9 tubes. In this procedure, the slices are freely floating in the physiological solution. Scintillation vessels made of polyethylene, having a total capacity of 5 ml, were used as wash-out tubes. The amount of radioactive tracer in each wash-out tube was measured (Packard Autogamma scintillation spectrometer) as well as that left in the slices at the end of the experiment.

The results of wash-out experiments are expressed in terms of a specific efflux rate R_t defined as $R_t = (C_{(t-\Delta t)} - C_t)/(C_{(t-\Delta t)} \cdot \Delta t)$, in which C_t is the $^{22}Na^+$ content of the slices at time t and $C_{(t-\Delta t)}$ is the content at time $(t - \Delta t)$. The stimulatory effect of an effector on the specific efflux rate is expressed in terms of Δ, a parameter equal to the difference between the average values of R_t measured during the 2 min before and the 2 min after the exposure to the effector. The values of both R_t and Δ, which have the dimension of min^{-1}, are independent of the amount of tissue used and of the actual amount of radioactive material released and are reproducible with a SD of ±15%.

RESULTS

L-Glu and other excitatory amino acids depolarize the neuronal membrane by increasing its permeability to Na^+ (6, 16, 18, 24). The enhanced permeability manifests itself by an increase in the fluxes of Na^+, influx and efflux, across the membrane. Because the efflux of ions is more convenient to measure than the

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Abbreviations: L-Asp, L-aspartate; Me-D-Asp, *N*-methyl-D-aspartate; L-Glu, L-glutamate; $Glu(OEt)_2$, diethyl glutamate.

influx, we have devised the method described above to monitor $^{22}Na^+$ efflux from preloaded brain slices. The specific rate of $^{22}Na^+$ efflux is used as a sensitive index of the interaction of excitatory amino acids with receptors controlling membrane permeability.

Na^+ Efflux from Striatal Slices. Fig. 1 shows the evolution of the specific $^{22}Na^+$ efflux rate with time. After 14 transfers, carried out to eliminate the bulk of the radioactivity present in the extracellular space, the specific efflux rate is stabilized around a value of 0.1 min^{-1}. If the slices are now transferred into solutions containing L-Glu or one of the other excitatory agents listed in Table 2, one observes a marked increase of the specific efflux rate, followed by a decrease. Fig. 1 illustrates the effects of L-Glu, at 0.3 and 3 mM, on the specific $^{22}Na^+$ efflux rate. Similar results are obtained when the slices are exposed to the effector at an earlier time than in the experiment shown in Fig. 1. However, in order to optimize the signal-to-noise ratio, the effector-stimulated $^{22}Na^+$ efflux rates are best measured at times when the bulk of the $^{22}Na^+$ present in the extracellular space has been removed. The tissue specificity of L-Glu-induced $^{22}Na^+$ efflux was investigated, and L-Glu was found to be unable to increase the rate of $^{22}Na^+$ efflux from slices of rat kidney, liver, and striated muscle.

Origin of the Excitatory Amino Acid-Induced Increase in Specific Efflux Rate. Theoretically, the excitatory amino acid-induced increase in $^{22}Na^+$ efflux rate may involve one or a combination of the following processes: (*i*) Activation of action potentials, resulting in an increase in Na^+ movements across the excitable membrane. (*ii*) Activation of Na^+/K^+-activated ATPase by the amino acid-induced depolarization. (*iii*) Activation of polysynaptic pathways, resulting in the release and subsequent interaction of various excitatory neurotransmitters with receptors triggering increases in membrane permeability to Na^+. (*iv*) Specific opening of Na^+ channels linked to excitatory amino acid receptors.

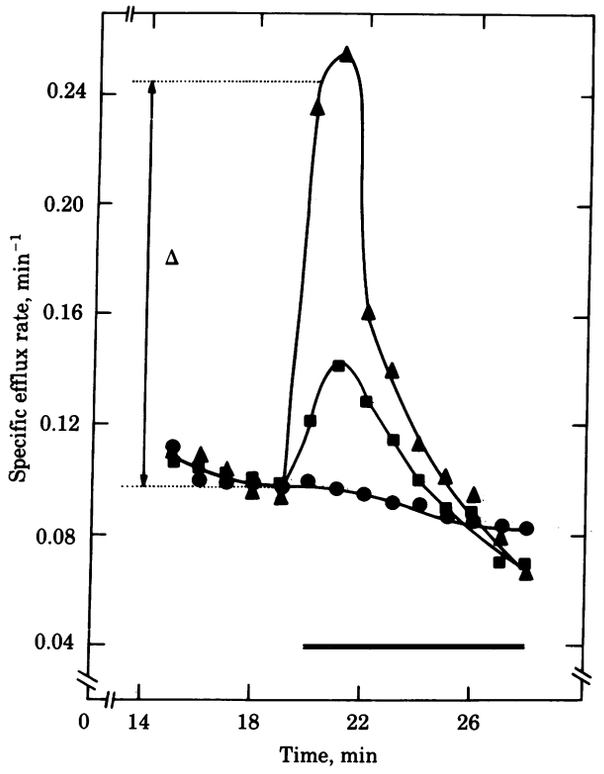

FIG. 1. Effect of L-Glu on specific $^{22}Na^+$ efflux rate from preloaded rat striatum slices. These experiments were conducted in a medium including from tube 15 onwards 0.1 μM tetrodotoxin, 0.2 mM $CaCl_2$, 4 mM $MgCl_2$. Transfers were carried out at the frequency of one transfer per min. The vertical arrow indicates the amplitude of the response (Δ) to 3 mM L-Glu. Each point represents a mean value from six experiments. The SD never exceeded ±15%. The black horizontal bar indicates the time of exposure to L-Glu at the following concentrations: 3 mM (▲), 0.3 mM (■). The basal efflux rate is represented by ●.

The possible contribution of each of the above processes was investigated and the results are presented in Table 1. Inspection of the values of Δ shows that this parameter does not vary under conditions preventing the activation of action potentials (0.1 μM tetrodotoxin), polysynaptic pathways (low $[Ca^{2+}]/[Mg^{2+}]$ ratio) and Na^+/K^+-activated ATPase (low temperature). The tetrodotoxin concentration of 0.1 μM was chosen because it was found to block the $^{22}Na^+$ efflux stimulated by 100 μM veratridine, a neurotoxin known to maintain the Na^+ action potential channel in an open state. Inhibition of the Na^+/K^+-activated ATPase was achieved by carrying out wash-out experiments at 15°C rather than at 37°C. The activity of Na^+/K^+-activated ATPase decreases to ⅓ for a 10°C temperature drop, and therefore its activity at 15°C should be reduced to about 10% of that at 37°C (25). As seen in Table 1, the temperature is the only parameter affecting the basal specific efflux rate. This result is expected because the Na^+/K^+-activated ATPase plays an important role in the turnover and removal of intracellular Na^+. The values of Δ, however, are not temperature dependent. The data presented in Table 1 strongly suggest that the excitatory amino acid-stimulated $^{22}Na^+$ effluxes originate from receptor-linked Na^+ channels.

To determine whether the L-Glu-stimulated increase in membrane permeability involves other ions besides Na^+, experiments similar to those described above were carried out by substituting $^{42}K^+$, $^{86}Rb^+$, $^{36}Cl^-$, or $^{51}Cr^{3+}$ for $^{22}Na^+$. L-Glu, tested at 1 mM, did not increase the specific efflux rate of $^{36}Cl^-$ or $^{51}Cr^{3+}$, but it caused a transient increase in the specific efflux rate of $^{42}K^+$ and $^{86}Rb^+$ from a basal value of 0.06 min^{-1} to a stimulated value of 0.08 min^{-1}.

Fig. 1 shows that the L-Glu-stimulated increase in specific $^{22}Na^+$ efflux rate is dose dependent. In Fig. 2, the values of Δ are plotted as a function of the logarithm of the bath concentrations of two excitatory effectors, L-Glu and kainate, an L-Glu analog. In both cases as well as with all the ligands tested, Δ reaches a maximal value of 0.18 min^{-1}. In view of the existence in brain of heterogeneous populations of excitatory amino acid receptors (8, 10, 11) that differ in their densities (26, 27), it is likely that the observed maximal value of Δ is not a reflection of full receptor occupancy. Among the factors that may limit the amplitude of Δ are the diffusion of the released $^{22}Na^+$ (resulting

Table 1. Effects of temperature, $[Ca^{2+}]/[Mg^{2+}]$ ratio, and tetrodotoxin concentration on the basal and L-Glu-stimulated specific $^{22}Na^+$ efflux rate from rat striatal slices

$[Ca^{2+}]/[Mg^{2+}]$	Tetrodotoxin, μM	Temp., °C	Specific $^{22}Na^+$ efflux rate, min^{-1}		
			Basal	Stimulated	Δ
1.5	—	37	0.101 ± 0.015	0.252 ± 0.033	0.151
1.5	0.1	37	0.093 ± 0.011	0.237 ± 0.021	0.144
0.05	—	37	0.103 ± 0.020	0.263 ± 0.016	0.160
0.05	0.1	37	0.096 ± 0.007	0.240 ± 0.042	0.145
0.05	0.1	15	0.067 ± 0.005	0.221 ± 0.032	0.154
0.05	0.1	23	0.087 ± 0.001	0.231 ± 0.018	0.151
0.002	0.1	23	0.083 ± 0.009	0.238 ± 0.012	0.155

L-Glu concentration: 5 mM. Δ is the difference between the averages of the basal and stimulated values of the specific $^{22}Na^+$ efflux rate. Rates are given as mean ± SD.

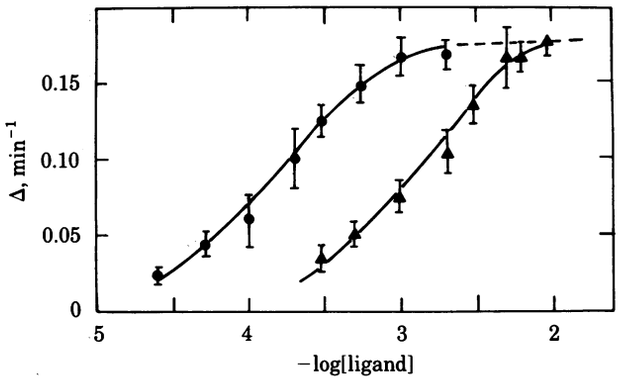

FIG. 2. Dose–response curves for L-Glu (▲) and for kainate (●). Each point represents the mean value for six experiments; bars indicate SD.

from permeability changes) from the extracellular space into the bathing medium and an effector-induced depletion of $^{22}Na^+$ from effector-sensitive cellular pools. These factors may play a role particularly at high effector concentrations. In order to minimize the contribution of the above factors, each effector was tested at concentrations giving rise to Δ values lower than 0.11 min^{-1} (see Fig. 2). Table 2 shows the relative efficacies of various L-Glu analogs. The efficacy was established by calculating, for each effector, the inverse ratio between the concentration of the effector eliciting a response Δ and the concentration of L-Glu eliciting the same response. Several amino acids and analogs, tested at the maximal bath concentration of 3 mM, were found to be devoid of agonist properties. They are also listed in Table 2.

Pharmacological Properties of Excitatory Amino Acid Receptors in the Rat Striatum. To investigate the pharmacological properties of the excitatory amino acid receptors in the rat striatum, we have tested several compounds reported in the literature for their antagonism of amino acid-induced excitation in the mammalian central nervous system (8–11). The following antagonists were tested: DL-α-aminoadipate, DL-α-aminosuberate, γ-D-glutamylglycine, 2-amino-5-phosphonovalerate, and diethyl glutamate.

Table 2. Relative efficacies of excitatory amino acids in inducing $^{22}Na^+$ efflux from preloaded rat striatum slices

N-Methyl-D-aspartate (Me-D-Asp)	50.0	D-Glu	1.2
		Piperidinedicarboxylate	1.2
DL-Homocysteate	11.0	L-Glu	1.0
Kainate	8.0	D-Asp	0.6
Quisqualate	6.4	L-Asp	0.6
Kainate methyl ketone* (28)	5.2	Dihydrokainate (30)	0.6
		L-Proline	0.2
Allokainate methyl ketone* (28)	4.6	L-Glutamate-γ-monohydroxamate	0.2
Carboxykainate* (29)	2.5		
Carboxyallokainate* (29)	2.3		

The efficacy was established by calculating for each ligand the inverse ratio between the bath concentration of the effector eliciting a response Δ and the concentration of L-Glu eliciting the same response (example: 1 mM L-Glu produced an effect Δ identical to that produced by 20 μM Me-D-Asp). All compounds were tested at least at two concentrations, 0.1 mM and 1 mM, and the more effective ones, Me-D-Asp and DL-homocysteate, also at 50 μM and 30 μM. The following compounds, tested at 3 mM, were found to be devoid of agonist properties: DL-α-aminopimelate, LL-α,ε-diaminopimelic acid, L-α-aminosuberate, diethyl glutamate, DL-α-methylglutamate, *N*-acetylkainate, γ-aminobutyrate, γ-D-glutamylglycine, 2-amino-5-phosphonovalerate, carbamoylcholine, glutarate, norepinephrine. The following compounds, tested at 1 mM, were found to be devoid of agonist properties: *N*-acetyl-L-aspartate, *N*-acetyl-DL-aspartate, L-glutamate-γ-hydrazide. The following compounds failed to affect the response to 0.5 mM L-Glu: 10 mM dithiothreitol, 10 μM substance P, 1 mM theophylline, 10 mM NaF.

* These compounds are derived from kainate and allokainate, respectively, by a formal replacement of the 4-isopropenyl side chain by an acetyl or a carboxyl group.

The competition experiments were carried out by exposing the slices to the antagonist for a total of 4 min, 2 min before and 2 min during the exposure to the agonist. Control experiments without antagonist were conducted in parallel. The agonists were used at concentrations giving rise to Δ values around 0.11 min^{-1}. The effect of each antagonist was expressed as the percent of the response obtained with the agonist alone. The results of these experiments are shown in Fig. 3. The effects of the antagonists on five different agonists were tested: Me-D-Asp, quisqualate, L-Asp, L-Glu, and kainate. Each agonist was found to be inhibited to a different extent by the various antagonists. For instance, Me-D-Asp was almost totally blocked by 2-amino-5-phosphonovalerate and γ-D-glutamylglycine, but the latter antagonized only partially the effects of L-Glu, kainate, and L-Asp and not at all the effects of quisqualate. With the exception of DL-α-aminosuberate, which antagonizes exclusively the effect of Me-D-Asp, no antagonist was found to be entirely selective in its actions.

The various antagonists, tested on D-glutamate (0.5 mM) and DL-homocysteate (30 μM) were found to block the response to these agonists in a pattern similar to that observed for Me-D-Asp, and at the same doses as those inhibiting the latter.

The profiles of antagonism shown in Fig. 3 may be interpreted by assuming the existence of heterogeneous populations of excitatory amino acid receptors. This assumption raises, then, the question of the identity of the natural ligands possibly acting at these sites. We have investigated this problem by studying the effects of amino acid antagonists on K^+-induced $^{22}Na^+$ effluxes from striatal slices. Exposure of the slices to high K^+ levels in the presence of 2 mM Ca^{2+} caused an increase in the specific $^{22}Na^+$ efflux rate. This effect was observed within the first 2 min of exposure to K^+ concentrations higher than 20 mM. At 40 mM K^+ (substituted for Na^+), a Δ value of 0.12 min^{-1} was obtained. This K^+-induced $^{22}Na^+$ efflux is Ca^{2+} dependent, because it is decreased to 1% by lowering the Ca^{2+} concentration to 0.02 mM. One can therefore assume that the K^+-induced $^{22}Na^+$ efflux is due to a K^+-evoked depolarization causing the release of endogenous excitatory neurotransmitters that act on subsynaptic receptors controlling the membrane permeability to Na^+. The amino acid antagonists were tested for their ability to block the Ca^{2+}-dependent K^+-induced $^{22}Na^+$ efflux. The results of these experiments are included in Fig. 3. One observes that the Ca^{2+}-dependent K^+-induced $^{22}Na^+$ efflux is antagonized to an almost similar extent by the same antagonists as those blocking the response to Me-D-Asp. It is therefore reasonable to assume that K^+ has triggered the release of a neurotransmitter displaying pharmacological properties similar to those of Me-D-Asp (as well as of D-Glu and DL-homocysteate) but different from those of other agonists, particularly L-Glu and L-Asp.

DISCUSSION

When striatal slices are exposed to L-Glu or other excitatory amino acids, the specific $^{22}Na^+$ efflux rate increases rapidly and then decreases. The results presented in Tables 1 and 2 and in Fig. 3 demonstrate that the increase in specific $^{22}Na^+$ efflux rate can be accounted for by the interaction of the excitatory effector with receptors controlling the membrane permeability. The decrease in the specific ion efflux rate observed after 2–3 min

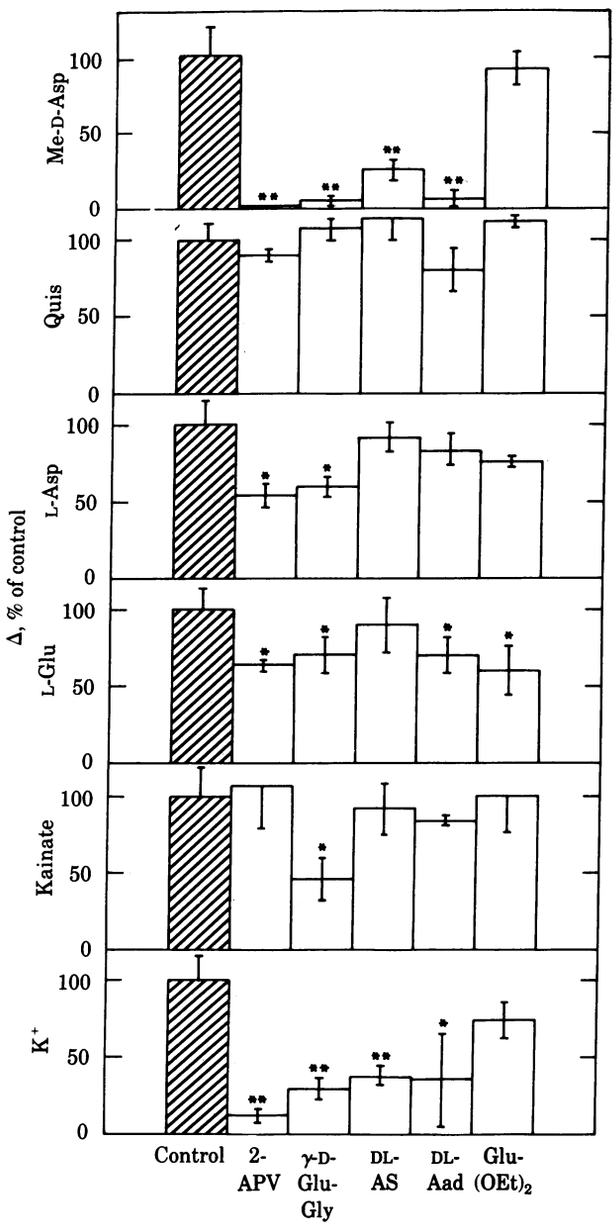

FIG. 3. Antagonism of the increase in specific $^{22}Na^+$ efflux rate produced by excitatory amino acids and high K^+ on $^{22}Na^+$-preloaded rat striatal slices. The agonists were tested at the following concentrations: Me-D-Asp, 30 μM (shown) and 500 μM; quisqualate (Quis), 100 μM; L-Asp, 1 mM; L-Glu, 0.5 mM; kainate, 100 μM. The response to these agonists was not changed by the presence of 0.02–2 mM Ca^{2+} and 1.2–10 mM Mg^{2+}, by 40 mM K^+, and 0.1 μM tetrodotoxin. The antagonists were used at the following concentrations: 2-amino-5-phosphonovalerate (2-APV), 1 mM; γ-D-glutamylglycine (γ-Glu-Gly), 1 mM; DL-α-aminosuberate (DL-AS), 1 mM; DL-α-aminoadipate (DL-Aad), 3 mM; diethyl glutamate [Glu(OEt)$_2$], 10 mM. Tested also at 50 μM, 2-amino-5-phosphovalerate was found to antagonize the response to 30 μM Me-D-Asp to the extent of 70%. The slices were depolarized by 40 mM K^+ (substituted for Na^+) in the presence of 2 mM Ca^{2+} and 1.2 mM Mg^{2+}. Tested at 50 μM, 2-amino-5-phosphonovalerate was found to block the effects of K^+ to the extent of 75%. Triplicate experiments were carried out simultaneously for each ligand concentration and were repeated at least twice. The vertical bar indicates SD, and significance compared to control was calculated by Student's *t* test: **, $P < 0.01$; *, $P < 0.05$.

of exposure of the brain slices to an excitatory effector could possibly be accounted for by a desensitization of receptors. However, no desensitization to L-Glu has yet been reported (14, 15, 24). A diffusion-limited wash-out of the tracer ions released into the extracellular space or a selective depletion of tracer ions in amino acid-sensitive cellular and dendritic pools are two alternative mechanisms that may account for the bell-shaped time dependence of the specific efflux rate. A compartmental analysis of the efflux data carried out according to Daniel (31) shows that a depletion process alone may fully account for the decrease in specific efflux rate (32). Because all the mechanisms proposed above may operate concomitantly, their individual contributions cannot be easily singled out. These processes underline, however, the main limitations of the $^{22}Na^+$ efflux assay. As excitatory amino acids interact with receptors controlling the membrane permeability to Na^+, a large receptor occupancy may lead to an exhaustion of $^{22}Na^+$ in sensitive pools before receptor saturation is reached. Therefore, full dose–response curves cannot be constructed and apparent effector–receptor affinities cannot be determined. It is nevertheless possible to classify excitatory amino acids according to their ability to increase the specific $^{22}Na^+$ efflux rate. It is clear, however, that the order of efficacy of excitatory amino acids established in the present $^{22}Na^+$ efflux assay cannot be viewed as a reflection of their relative affinities to receptors. Theoretically, mechanisms of amino acid uptake and degradation as well as the existence of diffusion barriers will affect the effector concentration, which will be different in the vicinity of the receptor than in the bathing fluid.

Because brain tissue possesses potent amino acid uptake systems effective in the millimolar range, for which no powerful inhibitors are yet available, it is not possible to assess quantitatively the role of uptake systems in the efficacy of excitatory amino acids to stimulate $^{22}Na^+$ efflux. Nevertheless, the order of efficacy established in this study by bath application is strikingly similar to that obtained in electrophysiological studies (4–6, 11). Moreover, it is noteworthy that in the latter no significant difference has been found in the order of potency of glutamatergic ligands established by iontophoresis or by bath application (5).

It may appear surprising that the stimulatory effects of L-Glu are observed in the millimolar range both in this study and in others (5, 12, 16). Although uptake mechanisms will modify the actual concentration of L-Glu at the receptor level, it is clear, that the effective concentration of L-Glu needed to activate the opening of the receptor-linked Na^+ channels will not be too far from the millimolar range. Because L-Glu is present in the cerebrospinal fluid at 30 μM (3), an apparent affinity of L-Glu for its receptor in the millimolar range should afford a margin of safety preventing fluctuations of the cerebrospinal fluid concentrations of L-Glu from giving rise to neuronal excitation.

By and large, the pharmacological data obtained in this study are in remarkable agreement with the literature and demonstrate the validity of the Na^+ flux assay. There are nevertheless, a few discrepancies that could possibly be accounted for by the fact that the studies were carried out on different tissues. In agreement with the results of Johnston *et al.* (4), dihydrokainate is found to be much less potent than kainate. However, the efficacies of the 3,4-*cis* isomers of the methyl ketone and carboxy derivatives of kainate are very similar to those of their respective 3,4-*trans* isomers (allokainate derivatives). This indicates, in contrast to an earlier suggestion (5), that the 3,4-*cis* stereochemistry is not essential for the activity of kainate derivatives.

Inspection of the patterns of antagonism established in this study confirms the existence of heterogeneous populations of excitatory amino acid receptors (8, 10, 11) and suggests the presence of at least four distinct receptors: a "Me-D-Asp receptor," a "quisqualate receptor," a "kainate receptor," and a "L-Glu/L-Asp receptor." Alternatively, it is possible to assume the ex-

istence of a single receptor with agonist sites allosterically regulated by the various antagonists. A distinction between the two propositions will require a study of the types of interactions between the various antagonists and the agonist binding sites. In the cat spinal cord, however, the existence of three types of receptors has been suggested, and no specific receptors have been assigned to L-Glu and L-Asp (11). The main discrepancies with our study concern the findings that the response of cat spinal neurons to L-Asp is strongly inhibited by 2-amino-5-phosphonovalerate and that the response to quisqualate can be partially blocked by $Glu(OEt)_2$ (11).

The possible existence of heterogeneous populations of excitatory amino acid receptors raises the question of the identity of their natural ligands. In the striatum, L-Glu is considered as a possible transmitter of the massive corticostriatal pathway (33–36). This suggestion is based mainly on the observation of an antagonism by $Glu(OEt)_2$ of the cortical excitation of striatal neurons (33). This has been supported by the finding that L-Glu is taken up by corticostriatal terminals and can be released upon K^+ depolarization (35, 36). In view of the results of this and other studies (7, 8) indicating the poor specificity and potency of $Glu(OEt)_2$ as an antagonist of amino acid excitation, the definite assignment of L-Glu as a major neurotransmitter in the striatum may not yet be totally warranted. In particular, the similitude of the patterns of antagonism of the responses to high K^+ levels and to Me-D-Asp (as well as to D-Glu and DL-homocysteate) but not to L-Glu or L-Asp strongly suggests the release of a neurotransmitter substance with different pharmacological properties than those of L-Glu or L-Asp. The fact that the different antagonists do not inhibit the K^+ and the Me-D-Asp depolarization to the same extent, as well as the finding of a small inhibition by $Glu(OEt)_2$, suggests that a release of L-Glu takes place concomitantly with the release of an Me-D-Asp-like substance. However, the contribution of L-Glu to the increase in $^{22}Na^+$ efflux is not as important as that of the Me-D-Asp-like substance. The suggestion of the release of a "Me-D-Asp-like substance" with neurotransmitter properties is supported by a growing number of reports showing that in other brain areas, Me-D-Asp antagonists depress both the synaptic and Me-D-Asp-evoked excitation (8, 10, 37, 38). The physiological role of a Me-D-Asp-like substance in the striatum and its chemical identity need now to be established.

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