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Classification and Properties of Acidic Amino Acid Receptors in Hippocampus

II. Biochemical Studies Using a Sodium Efflux Assay

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SUMMARY

The properties of excitatory amino acid receptors in hippocampal slices were analyzed using agonist-induced stimulation of ^{22}Na efflux rate. Several amino acids (L- and D-glutamate, *N*-methylaspartate) produce progressively smaller responses upon successive applications, whereas D,L-homocysteate does not. Several lines of evidence suggest that depletion of an intracellular pool of ^{22}Na is not responsible for the apparent desensitization. Addition of the amino acids in the presence of an antagonist does not affect the response of the slices to subsequent applications, indicating that desensitization is dependent upon the interaction of the agonist with its receptor. The antagonist D-α-aminoadipate discriminates between various excitatory amino acids, completely blocking the responses to *N*-methylaspartate, D-glutamate, and D,L-homocysteate; partially antagonizing those of quisqualate and kainate; and being without effect on L-glutamate. The order of potency of several excitatory amino acids on the stimulation of ^{22}Na efflux rate in hippocampal slices is highly correlated with their relative effects measured with electrophysiological techniques, but does not correlate with their relative potencies to inhibit [^{3}H]glutamate binding to hippocampal membranes. The similarities in the properties of excitatory amino acid receptors evidenced with the ^{22}Na efflux assay or with the electrophysiological approach in the *in vitro* hippocampal slice preparation indicate that the same receptors are sampled by the two techniques. The results are discussed in terms of a classification of these receptors into four different groups: a synaptic receptor, activated by D,L-homocysteate (tentatively defined as a G_1 receptor), an extrasynaptic glutamate receptor (defined as a G_2 receptor), an *N*-methylaspartate receptor, and a kainate receptor.

INTRODUCTION

Electrophysiological studies of excitatory amino acid receptors in vertebrate nervous systems have defined several classes of receptors, primarily on the basis of agonist-antagonist interactions (see refs. 1 and 2 for reviews). More recently, we differentiated four types of receptors according to their degree of desensitization following successive applications of agonists as well as their relative sensitivities to different antagonists (3). Thus we identified a G_1 receptor (thought to be a synaptic glutamate receptor), a G_2 receptor (an extrasynaptic glutamate receptor), an *N*-methylaspartate receptor, and a kainate receptor. The classification arrived at from these electrophysiological studies does not accord well with that deduced from the binding of radiolabeled ligands to purified membranes (see ref. 4 for a review). Binding sites that discriminate between glutamate (5–9), aspartate (10, 11), and kainate (12) have been identified, but their pharmacological profiles do not correspond with the results of physiological studies of drug interactions.

Recently, Luini *et al.* (13) used the stimulation of ^{22}Na efflux in striatal slices elicited by various excitatory amino acids to define biochemically several classes of amino acid receptors. In the present study we compare the properties of similarly defined amino acid receptors in slices from the hippocampus, a structure in which glutamate and/or aspartate is likely to be the neurotransmitter of several intrinsic and extrinsic systems (14), with those arrived at from electrophysiological studies in the *in vitro* hippocampal slice preparation (3) or by the binding of [^{3}H]glutamate to hippocampal membranes (9). The results strengthen the idea that four classes of excitatory amino acid receptors are found in hippocampus

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and that the [^{3}H]glutamate binding site previously characterized in hippocampal membranes is associated with a synaptic receptor (G_1).

MATERIALS AND METHODS

Sprague-Dawley rats (150–250 g) were used in all of the studies. ^{22}Na (143 Ci/g; 200 μCi/ml) was purchased from the Radochemical Center, Amersham (Arlington Heights, Ill.), and [^{3}H]glutamate (33 Ci/mmole) from ICN (Irvine, Calif.). L-Glutamic, D-glutamic, D,L-homocysteic, quisqualic, *N*-methyl aspartic, kainic, quinolinic, L-aspartic, and D-α-amino adipic acids were obtained from Sigma Chemical Company (St. Louis, Mo.). D,L-Aminophosphonobutyric acid was purchased from Calbiochem (La Jolla, Calif.).

Measurement of ^{22}Na efflux in hippocampal slices. To measure the stimulation of ^{22}Na efflux induced by excitatory amino acids, we used the procedure described by Luini *et al.* (13) and Teichberg *et al.* (15) with minor modifications as described below. Rats were killed by decapitation, their brains were rapidly removed, and the hippocampi were dissected. Transverse sections (400 μm thick) were cut with a McIllwain tissue chopper and preincubated at 33° in an oxygenated physiological medium containing (final concentration) 124 mM NaCl, 3.33 mM KCl, 1.25 mM KH_2PO_4, 2.41 mM $MgSO_4$, 2.45 mM $CaCl_2$, 10 mM D-glucose, and 25 mM Tris, the pH being adjusted to 7.3 with HCl. After 40 min the slices were washed three times with freshly oxygenated medium and incubated for 30–40 min at 33° in 1.0 ml of physiological medium containing ^{22}Na at a concentration of 8 μCi/ml. After this loading period, slices (two or three per tube) were placed into basket-shaped sieves and transferred every minute through a series of 16–30 tubes, each containing 1.0 ml of nonradioactive physiological solution saturated with O_2, at room temperature. The drugs to be tested were included in some of the tubes as indicated in the various figures. The amount of radioactivity in each washout tube was measured, as well as that left in the slices at the end of the experiment. The results were then described in terms of a specific efflux rate, k, defined as $k_t = [C_{(t-\Delta t)} - C_t/(C_{(t-\Delta t)} \cdot \Delta t]$, where C_t represents the radioactivity in the slices at time t and $C_{t-\Delta t}$ the content at time $t-\Delta t$. The stimulatory effect of an effector on the specific efflux rate was expressed in terms of an index, $I = \sum_t (k_t - \bar{k})/\bar{k}$, where k_t represents the efflux rate in the presence of the effector and $\bar{k}$ the mean of the efflux rates for the 2 min before the exposure to the effector (see Fig. 1 for a schematic explanation).

[^{3}H]Glutamate binding to hippocampal membranes. Although most of the binding data reported in the present study have been previously published (9), when needed, [^{3}H]glutamate binding to hippocampal membranes was assayed as previously described (16).

RESULTS

Quantification of the effects of various excitatory amino acids. Figures 1 and 2 illustrate the method we used to quantify the effects of various compounds on the specific ^{22}Na efflux rate. Our procedure differs from that used by Luini *et al.* (13) in that we summate the effects of each agonist for the duration of its application, rather than limiting the analysis to the first 2 min. In other words, our index I represents the area (normalized to the baseline measured before effector application) under the curve of efflux rate as a function of time, during the period of application. Some interesting features emerge from this procedure. Thus, whereas there is a linear relationship between the stimulatory effect, as reflected by our index I, and time in the case of D,L-homocysteate (at least for up to 5–6 min of application), the effect of L-glutamate reaches a plateau after 2–3 min of application, after which it starts to decrease (Fig. 1). Conversely,

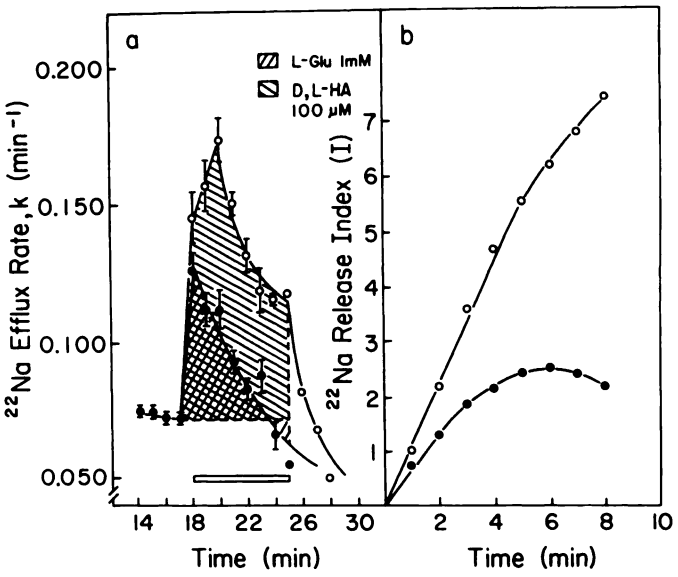

FIG. 1. *Effects of L-glutamate (L-Glu) and D,L-homocysteate (D,L-HA) on the ^{22}Na efflux rate in hippocampal slices*

After loading slices with ^{22}Na for 40 min at 33°, slices were transferred every minute in nonradioactive medium as described under Materials and Methods. At the indicated times (*horizontal bar*), L-glutamate (1 mM) or D,L-homocysteate (100 μM) was included in the washout tube for increasing periods of time.

a. The results are expressed in terms of a specific efflux rate, k, determined as described under Materials and Methods, and are means ± standard error of the mean of six to eight experiments. The *hatched area* reflects the interpretation of the release index I calculated as described under Materials and Methods and which approximately represents a normalized value of this area.

b. The release index for L-glutamate (●) or D,L-homocysteate (○) is calculated as a function of the time of the agonist application (mean of six to eight experiments).

in the case of *N*-methylaspartate and even more noticeably with kainate, there is a lag before the onset of the agonist effect (Fig. 2), followed by a linear increase in I as a function of time.

Desensitization and cross-desensitization to various agonists. Since desensitization of glutamate receptors occurring during the application of the amino acid could be responsible for the nonlinearity between the stimulatory effect and time, we determined the effects of successive applications of various excitatory amino acids on the specific ^{22}Na efflux rate. As shown in Fig. 3a and b, the effects of L- as well as those of D-glutamate are markedly reduced following a first application (also see Table 1). On the other hand, the response to D,L-homocysteate is not significantly affected by prior administration of this amino acid (Fig. 3c; Table 1). *N*-Methylaspartate receptors also exhibit apparent desensitization (Table 1); the effects of successive applications of kainate could not be tested because of the very long-lasting increase in ^{22}Na efflux rate elicited by this compound. Desensitization to D-glutamate requires the interaction of this agonist with its receptor, since the simultaneous application of D-α-aminoadipate, which almost totally blocks the effect of D-glutamate on ^{22}Na efflux, prevents desensitization (Fig. 3d; Table 1). In order to test the possibility that the

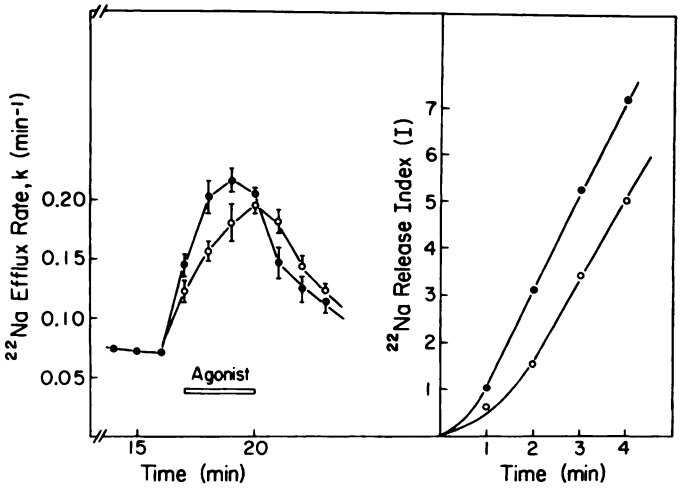

FIG. 2. *Effects of N-methylaspartate and kainate on the ^{22}Na efflux rate in hippocampal slices*

Same data as in legend to Fig. 1. ●, *N*-Methylaspartate (50 μM); ○, kainate (100 μM). Results are means (right) or means ± standard error of the mean (left) of four to six experiments.

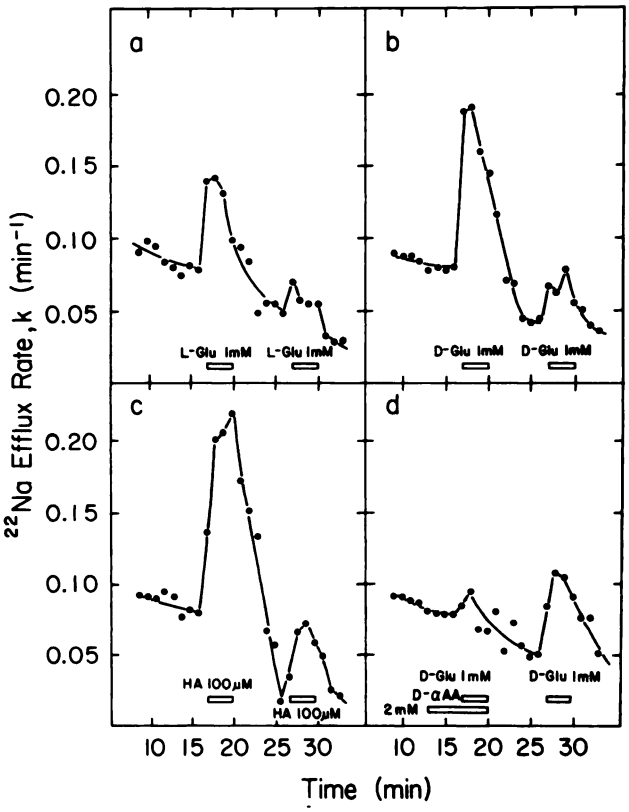

FIG. 3. *Effects of successive application of L-glutamate (L-Glu), D-glutamate (D-Glu), or D,L-homocysteate (HA) on the ^{22}Na efflux rate in hippocampal slices*

^{22}Na efflux rate in hippocampal slices was determined as described under Materials and Methods. The same concentration of various amino acids was included in the medium following a 6-min washout period after a 4-min application. In d, 2 mM D-α-aminoadipate (*D-αAA*) was included during and for 4 min before the first application of 1 mM D-glutamate. Results are means of three or four experiments.

apparent desensitization to various agonists merely reflects the exhaustion of some ^{22}Na pools [as suggested by Teichberg *et al.* (15)], we measured the effects of a second application of various agonists following a first application of a different agonist (Fig. 4; Table 1). Following a single application of L-glutamate, the effects of D,L-homocysteate, *N*-methylaspartate, and kainate were not significantly different from those determined in the absence of a previous application of an agonist (in separate experiments, we found that the effect of a given agonist is the same whether applied at 17 min or at 27 min during the washout period). Following a first application of D,L-homocysteate, L-glutamate still elicited a stimulation of ^{22}Na efflux rate comparable to the effect seen after a first application. Finally, the response to L-glutamate was only slightly reduced (Table 1) when the amino acid was applied to slices previously treated with *N*-methylaspartate.

Effect of D-α-aminoadipate on the responses elicited by various agonists. The excitatory amino acid antagonist α-aminoadipate has been shown to block synaptic transmission and to affect differentially the depolarizing

TABLE 1

Desensitization and cross-desensitization between various excitatory amino acids

^{22}Na efflux rate in hippocampal slices was determined as described under Materials and Methods. The first application of an amino acid for 4 min was followed 6 or 8 min later (see legends to Figs. 3 and 4) by the application of the same or a different amino acid for 4 min. The results are expressed in terms of the ^{22}Na release index, calculated as described under Materials and Methods; they are the means ± standard error of the mean of four to six experiments or the means of two experiments which differed by less than 15%.

Amino acid	First application	Amino acid	Second application
L-Glutamate, 1 mM	2.38 ± 0.12	L-Glutamate, 1 mM	0.53 ± 0.34
D,L-Homocysteate, 100 μM	5.69 ± 0.11	D,L-Homocysteate, 100 μM	5.81 ± 0.41
L-Glutamate, 1 mM	2.38 ± 0.12	D,L-Homocysteate, 100 μM	6.53 ± 0.84
D,L-Homocysteate, 100 μM	5.53	L-Glutamate, 1 mM	2.34
L-Glutamate, 1 mM	2.68 ± 0.24	*N*-Methylaspartate, 50 μM	6.04 ± 0.49
		Kainate, 100 μM	5.81 ± 0.11
N-Methylaspartate, 50 μM	6.26 ± 0.40	NT[a]	
Kainate, 100 μM	5.03 ± 0.24	NT	
N-Methylaspartate, 20 μM	4.77	*N*-Methylaspartate, 20 μM	1.93
		L-Glutamate, 1 mM	1.88
D-Glutamate, 1 mM	5.00	D-Glutamate, 1 mM	1.45
D-Glutamate, 1 mM, + D-α-aminoadipate, 2 mM	0.08	D-Glutamate, 1 mM	5.17

[a] NT, Not tested.

actions of various agonists in the hippocampal slice preparation (3). Similarly, D-α-aminoadipate differentially influences the changes in ^{22}Na efflux rate elicited by various agonists (Fig. 5). Thus, while it virtually blocks the effects of D-glutamate, D,L-homocysteate, and *N*-methylaspartate, it only slightly reduces the response to kainate and quisqualate (by about 30–40%) and does not modify the effect of L-glutamate. The effects of D-α-aminoadipate are dose-dependent, with an IC_{50} of about 0.23 mM when tested against 100 μM D,L-homocysteate (data not shown). Although D,L-aminophosphonobutyrate is presumed to act as an antagonist and has been shown to block synaptic transmission in the hippocampal slice preparation (3, 17), it produces a marked increase in ^{22}Na efflux rate (Fig. 6), making it difficult to assess its antagonistic properties.

Comparison of the potency of various excitatory amino acids to stimulate ^{22}Na efflux and to depolarize hippocampal slices. The use of hippocampal slices allowed us to compare on the same preparation the relative potency of various excitatory amino acids to stimulate ^{22}Na efflux rate and to depolarize hippocampal neurons. We previously described our method for quantifying the physiological effects of various agonists on CA_1 neurons

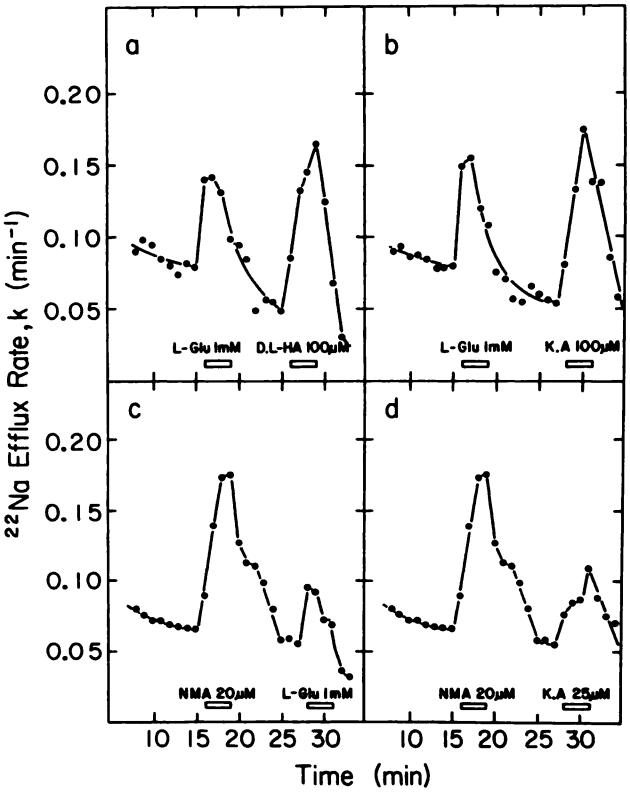

FIG. 4. *Effects of various amino acids following the application of a different agonist on the ^{22}Na efflux rate in the hippocampal slices*

^{22}Na efflux rate in hippocampal slices was determined as described under Materials and Methods.

a. D,L-Homocysteate (*D.L-HA*) (100 μM) was applied for 4 min 6 min following the application of L-glutamate (*L-Glu*) (1 mM) for 4 min.

b. Kainate (*K.A*) (100 μM) was applied for 4 min 8 min after the application of L-glutamate for 4 min.

c and d. L-Glutamate (1 mM) or kainate (25 μM) was applied for 4 min 8 min after the application of *N*-methylaspartate (*NMA*) (20 μM) for 4 min. Results are means of three experiments.

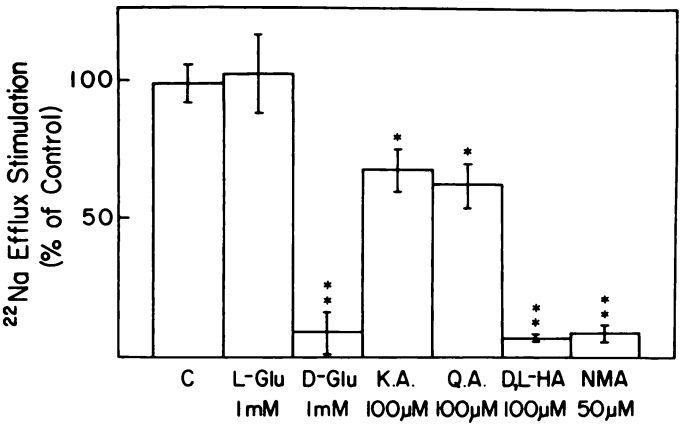

FIG. 5. *Effects of D-α-aminoadipate on the stimulation of ^{22}Na efflux rate elicited by various excitatory amino acids in hippocampal slices*

The stimulatory effect of various excitatory amino acids on the ^{22}Na efflux rate in hippocampal slices was determined as described under Materials and Methods. D-α-aminoadipate (2 mM) was present during and for 4 min before the 4-min application of the agonists. Results are expressed as percentage of the release index measured in the absence of the antagonist and are means ± standard error of the mean of four to six experiments. * $p < 0.01$; ** $p < 0.001$ (Student's *t*-test). *C*, Control; *L-Glu*, L-glutamate; *D-Glu*, D-glutamate; *K.A.*, kainate; *Q.A.*, quisqualate; *D,L-HA*, D,L-homocysteate; *NMA*, *n*-methylaspartate.

(3). Figure 7 shows concentration-response curves for D,L-homocysteate on both the stimulation of ^{22}Na efflux rate and on the depolarizing effect on CA_1 pyramidal cells. The two curves are totally superimposable, with an EC_{50} for D,L-homocysteate of about 50 μM and a maximal effect obtained at a concentration of 100–200 μM. Also shown in Fig. 7 is a concentration-response curve for L-

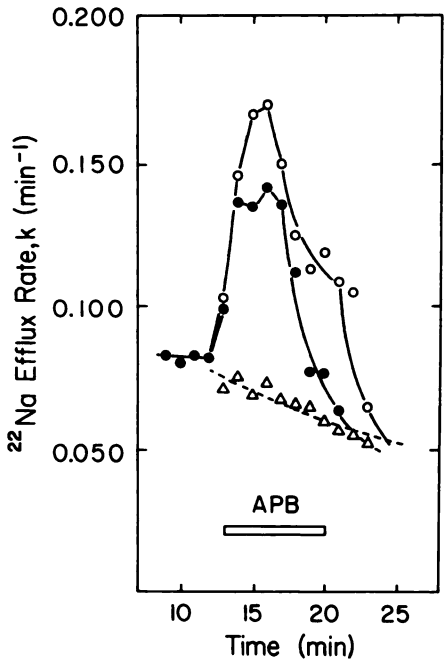

FIG. 6. *Effects of 2-aminophosphonobutyrate (APB) on the ^{22}Na efflux rate in hippocampal slices*

^{22}Na efflux rate in hippocampal slices was determined as described under Materials and Methods. Results are means of three or four experiments. △, APB 0; ●, APB 1 mM; ○, APB 2 mM.

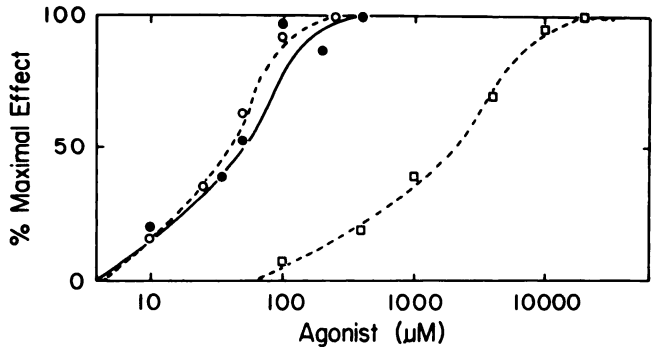

FIG. 7. *Concentration-response curve for* D,L *-homocysteate and* L *-glutamate on the stimulation of* ^{22}*Na efflux rate in hippocampal slices*

^{22}Na efflux rate in hippocampal slices and the amino acid-induced stimulatory effect (○, □) were determined as described under Materials and Methods. The D,L-homocysteate-induced depolarization of CA_1 neurons (●) was determined as published elsewhere (3). Results are expressed as percentage of the maximal effect and are means of four to six experiments.

glutamate on the stimulation of ^{22}Na efflux rate, which indicates that the threshold for the effect is about 0.1 mM, the EC_{50} about 2 mM, and the maximal response obtained at 10–20 mM. The relative potency on both parameters of a variety of excitatory amino acids is listed in Table 2. Although some differences are found, there is a positive correlation between the two parameters ($r = 0.92$; $p < 0.05$). Table 2 also indicates the relative potency of these agonists to inhibit [^{3}H]glutamate binding to hippocampal membranes. No apparent correlation exists between this parameter and the relative potencies to depolarize hippocampal slices or to stimulate ^{22}Na efflux.

TABLE 2

Comparison of the relative potency of various excitatory amino acids to stimulate ^{22}*Na efflux, depolarize hippocampal slices, and inhibit [*^{3}*H]glutamate binding to hippocampal membranes*

The stimulation of ^{22}Na efflux rate elicited by various excitatory amino acids was determined as described under Materials and Methods. The depolarization elicited by the same amino acids on CA_1 neurons in the *in vitro* hippocampal slice preparation was determined as published elsewhere (3). The inhibition of [^{3}H]glutamate binding to hippocampal synaptic membranes was determined as published elsewhere (4).

For the first two columns the relative potency represents the inverse ratio between the concentration of the agonist required to elicit a response and the concentration of L-glutamate eliciting the same response. For [^{3}H]glutamate binding, the relative potency represents the ratio of the K_I of the various agonists to the K_d of [^{3}H]glutamate.

	^{22}Na efflux	Depolarization	Inhibition of [^{3}H]glutamate binding
L-Glutamate	1	1	1
n-Methylaspartate	100	200	0
Kainate	40	400	0
D,L-Homocysteate	40	20	0.5
Quisqualate	15	200	4
Quinolate	9	30	0
L-Aspartate	2	1	0.13
D-Glutamate	2.5	2	0.08
Cysteine sulfinate	0.5	0.25	ND[a]

[a] ND, Not determined.

DISCUSSION

The present results confirm and extend the previous reports by Luini *et al.* (13) and Teichberg *et al.* (15) demonstrating the utility of the ^{22}Na efflux assay in defining excitatory amino acid receptors. Our results obtained using hippocampal slices are in very close agreement with theirs for striatal slices. For instance, the order of potency of various agonists to stimulate ^{22}Na efflux in hippocampal slices is almost the same as that for striatal slices. Similarly, the antagonism by D-α-aminoadipate of the effects of a variety of excitatory amino acids is virtually identical in hippocampal and striatal slices. The small differences between the earlier results and ours may be due to the different way in which we chose to express the effects of the agonists. While Luini *et al.* (13) and Teichberg *et al.* (15) considered the changes in efflux rate during the first 2 min of application, we summated the effects for the whole period of application. This difference has two obvious implications: in the case of a slowly acting agonist, the earlier authors tend to underestimate effects, whereas we would underestimate the effects of an agonist which desensitizes during its application. Plotting our index of stimulation as a function of time indeed revealed marked differences between the time course of various agonists. Thus, L-glutamate appears to fall under the latter category whereas kainate belongs to the former. We also reach a different conclusion on the question of desensitization. Luini *et al.* (13) and Teichberg *et al.* (15) interpreted the bell-shaped curve of most of the agonists on the basis of the exhaustion of an intracellular pool of labeled sodium ions. Several arguments suggest that desensitization of the effects of those agonists is more likely to be responsible for such a curve. First, using successive applications of the same agonist, we found both non-desensitizing (D,L-homocysteate) and desensitizing (L- and D-glutamate, *N*-methylaspartate) agonists. Results of the cross-desensitizing experiments, in which an agonist was applied after a first application of a different agonist, strongly indicate that exhaustion of an intracellular pool of sodium ions is not likely to be responsible for the apparent desensitization phenomenon. In order to maintain such an explanation, one would have to postulate that each of these amino acids acts on a separate intracellular pool of sodium ions (this seems very unlikely since we have found no evidence of additivity of the effects of various excitatory amino acids). Similarly, the absence of cross-desensitization between various agonists excludes the possibility that desensitization merely represents an accumulation of potassium ions in the extracellular space. This also makes it unlikely that the second response to an agonist is underestimated because of a different value for the basal release rate. Finally, as discussed later, electrophysiological experiments using the hippocampal slice preparation indicate the existence of desensitization to certain excitatory amino acids (3, 18). The apparent desensitization of D-glutamate requires the interaction of the agonist with its receptor, since the simultaneous presence of the antagonist D-α-aminoadipate, which almost totally blocks the ^{22}Na efflux elicited by D-glutamate, prevents desensitization. This would also rule out the possibility

that desensitization represents a difference in the availability of the agonist due to modification in enzymatic degradation or uptake process, since D-α-aminoadipate interacts only marginally with glutamate uptake (19).

The use of hippocampal slices prepared under conditions similar to those employed in electrophysiological experiments allowed us to compare two quite different measures of the properties of excitatory amino acid receptors. The time-course of stimulation of the ^{22}Na efflux produced by the agonists correlates well with that for depolarization of hippocampal neurons. Kainic acid has been found to produce a slowly appearing but long-lasting depolarization of CA_1 neurons (20), and this agrees with its effect on the ^{22}Na efflux. We also found that the depolarizing effects of L-glutamate exhibit a tendency to extinguish following an application period exceeding 4 or 5 min (18); this agrees well with the gradual decrease of its effect on the ^{22}Na efflux rate. The dose-response curves of D,L-homocysteate on both depolarization and ^{22}Na efflux are exactly superimposable, which suggests that the opening of sodium channels is responsible for the depolarization induced by this amino acid. The same amino acids that exhibit the phenomenon of desensitization of their physiological responses also exhibit desensitization of the stimulation of ^{22}Na efflux. Thus L- and D-glutamate as well as *N*-methylaspartate exhibit the desensitization phenomenon, whereas D,L-homocysteate does not. Similarly, cross-desensitization experiments suggest that several classes of noninteractive excitatory acid receptors exist. Moreover, the order of potency for several excitatory amino acids on the depolarization of CA_1 neurons parallels relatively closely the order of potency on the stimulation of ^{22}Na efflux rate in hippocampal slices. Finally, the effects of the antagonist D-α-aminoadipate on various excitatory amino acids are similar on both parameters. Whereas D-α-aminoadipate blocks the effects of D,L-homocysteate, D-glutamate, and *N*-methylasparate, it does not block the effect of L-glutamate.

Thus in terms of time courses, dose-response curves, desensitization, relative potencies of agonists, and pharmacological properties, the electrophysiological and sodium flux assays are comparable. The biochemical procedure should therefore serve as a valuable tool in amino acid pharmacology, particularly in light of its relative simplicity. For instance, the agonist effect of 2-aminophosphonobutyrate might relate to the depolarizing effect of this compound observed in various preparations (21).

Analysis of the properties of the sodium flux responses to excitatory amino acids in conjunction with their physiological effects (3) suggests that the hippocampus contains at least four classes of excitatory amino acid receptors: (a) a receptor (tentatively defined as a G_1 receptor) that is stimulated by homocysteic acid, does not desensitize, and is blocked by D-α-aminoadipate; (b) a receptor (defined as a G_2 receptor) that is stimulated by L-glutamate, desensitizes, and is not blocked by D-α-aminoadipate, (c) a receptor for *N*-methylaspartate that desensitizes and is blocked by D-α-aminoadipate, and (d) a non-desensitizing kainate receptor that is not blocked by D-α-aminoadipate. However, it must be pointed out that this classification, although adequate to account for most of the present evidence, needs to be tested with a larger number of agonists and antagonists.

The G_1 receptor is a reasonable candidate for the trasmitter receptor for the Schaffer-commissural pathway in hippocampus. Synaptic responses in this pathway are blocked by D-α-aminoadipate and do not cross-desensitize with L-glutamate or *N*-methylaspartate, two important properties that are shared by the homocysteic acid-G_1 receptor. In addition, potassium-induced stimulation of ^{22}Na efflux rate probably involves release of the endogenous transmitter and subsequent stimulation of the postsynaptic G_1 receptor, since it is suppressed by removal of calcium and decreased following the elimination of large numbers of synaptic terminals [ref. 13 and the following paper (22)]. The time course of potassium-induced stimulation of ^{22}Na efflux rate parallels very closely that of homocysteic acid, being linear for up to 6 min after application (data not shown). The G_1 site can be presumed to be postsynaptic since the effects of homocysteic acid are not diminished by removal of calcium or elimination of large numbers of postsynaptic endings [see the following paper (22)]. In light of this evidence, we propose the G_1 receptor as a candidate for the receptor of the endogenous transmitter.

The sodium-independent [^3H]glutamate binding site previously identified in hippocampal membranes is strongly inhibited by D-α-aminoadipate and homocysteic acid but does not recognize *N*-methylaspartate or kainate. The site is highly purified in junctional membranes (10) and, as shown in the following paper of this sequence (22), it has a postsynaptic locus. These characteristics suggest that this site forms part of the G_1 receptor ionophore complex.

The major problem with the above idea is that exogenously applied L-glutamate clearly does not interact with the synaptic receptor; thus the glutamate response in slices can be blocked (by desensitization) without affecting synaptic transmission, whereas transmission is inhibited by drugs (α-aminoadipate and 2-aminophosphonobutyrate) that have no effect on the physiological or biochemical responses to exogenous L-glutamate. Conceivably, the existence of very efficient uptake systems might prevent the exogenously applied L-glutamate from reaching the synaptic receptor at concentrations high enough to stimulate them. Moreover, the inhibition of [^3H]glutamate binding to hippocampal membranes by sodium ions (9) suggests that, in the normal environment of the synapse, the synaptic receptor might be in a conformational state with a low affinity for L-glutamate. Some clues about the regulation of these various classes of receptors are presented in the accompanying paper (22).

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