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CLASSIFICATION AND PROPERTIES OF ACIDIC AMINO ACID RECEPTORS IN HIPPOCAMPUS

I. Electrophysiological Studies of an Apparent Desensitization and Interactions with Drugs which Block Transmission[1]

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Abstract

The existence of multiple receptors for acidic amino acids has been proposed by several authors mainly on the basis of the different sensitivities of amino acid agonists to various antagonists. In the experiments described in the present paper we used two quite different experimental paradigms to define these receptors in slices of rat hippocampus and to investigate their possible relationships with the receptors for the synaptic transmitter(s) in the Schaffer-commissural pathway. First, we tested whether desensitization of the physiological responses to a given amino acid (produced by repetitive application of the compound) changed either the effects of other amino acids or the synaptic field potentials. Second, the antagonistic properties of α-aminoadipate (AA) and aminophosphonobutyric acid (APB), two drugs which block synaptic transmission, were measured on the physiological responses produced by perfused amino acids. In no case did we find that desensitization influenced evoked potentials, suggesting that the receptors which exhibit this property are distinct from those for the endogenous transmitter. Combining this finding with the relative sensitivities of perfused agonists to APB and AA, we classified excitatory amino acid receptors in the hippocampus into at least four distinct types: (*1*) an NMA receptor, activated by *N*-methyl aspartate (NMA) and D-glutamate, which exhibits desensitization and is blocked by AA but not by APB; (*2*) a kainate receptor which does not desensitize and is not blocked by AA or APB; (*3*) a L-glutamate/aspartate receptor which desensitizes and is not blocked by AA or APB (tentatively defined as a G_2 receptor); and (*4*) a receptor that does not desensitize, is activated by D,L-homocysteate, and is blocked by AA and APB (tentatively defined as a G_1 receptor). In view of the cross-desensitization between the G_2 and NMA sites, we propose that these two receptors share sodium permeability channels and are located extrasynaptically. The kainate receptor also appears to be distinct from the synaptic receptor, and the G_1 site is a reasonable candidate for the transmitter receptor. The significance of these results to the hypothesis that glutamate and/or aspartate serve a transmitter role in hippocampus is discussed.

An increasing number of biochemical and electrophysiological studies suggest that L-glutamate and L-aspartate are excitatory neurotransmitters in mammalian central nervous system (DiChiara and Gessa, 1980; Roberts et al., 1980; Watkins and Evans, 1981, for reviews). Both release and uptake have been demonstrated in defined synaptic systems (Wieraszko and Lynch, 1979; Baughman and Gilbert, 1980), and exogenous glutamate produces physiological effects which at least partially mimic those which accompany excitatory postsynaptic potentials (Hackett et al., 1979). However, it has proven difficult to satisfactorily relate a receptor for a particular amino acid to that used by an endogenous neurotransmitter. Based upon the various sensitivities of excitatory amino acids (i.e., amino acids that produce an increase in cell firing and/or a decrease of resting membrane potential) to a variety of antagonists, the existence of three types of receptors has been proposed: (*1*) an NMDA receptor stimulated by *N*-methyl-D-aspartate (NMDA) and blocked by aminophosphonovalerate, (2)

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a quisqualate receptor blocked by glutamate diethylester (GDEE), and (*3*) a kainate receptor blocked by γ-D-glutamylglycine (McLennan, 1981; Watkins and Evans, 1981). Most of the work leading to this classification used spinal cord preparations, and it is not yet clear if this schema can be extended to other CNS regions.

Another approach which has been used to discriminate various types of receptors consists of desensitizing the responses to a category of agonists by repetitive applications and determining the resulting effect on synaptic responses or on physiological effects of other agonists. Using this paradigm, Takeuchi and Takeuchi (1964) showed that desensitization of the responses to exogenous glutamate resulted in a parallel decrease in synaptic potentials at the crayfish neuromuscular junction. A similar approach was used by Kelly and Gage (1969) and Anderson et al. (1978) in various invertebrate preparations. Comparable studies have not been performed in the mammalian CNS, possibly because of the general assumption that desensitization to glutamate does not occur. However, in work using rat hippocampus, a structure in which glutamate has been proposed to be the neurotransmitter of several extrinsic and intrinsic pathways (Storm-Mathisen, 1977), we found that successive applications of glutamate caused an apparent desensitization of glutamate receptors. Moreover, this desensitization was not accompanied by a modification of synaptic responses, a finding which suggested the existence of glutamate receptors distinct from the synaptic receptors (Fagni et al., 1983). In the present study we extend these observations by examining desensitization and cross-desensitization to a variety of excitatory amino acids. This approach is then combined with the use of selective antagonists to further discriminate various categories of receptors. We suggest that four receptors can be differentiated and that one of these has the characteristics of the synaptic receptor of the Schaffer-commissural pathway.

Materials and Methods

In vitro hippocampal slices were prepared from adult male Sprague-Dawley rats and incubated as described previously by Dunwiddie and Lynch (1978). Briefly, rats were decapitated, and the brain was rapidly removed and placed in ice-cold medium. Hippocampi were dissected, cut in 400-μm transverse slices, and placed in a recording chamber at a temperature of 34 to 35°C. The medium in the chamber had the following composition (mM): NaCl, 124; KCl, 3.33; KH_2PO_4, 1.25; $MgSO_4$, 1.33; $CaCl_2$, 3.13; $NaHCO_3$, 25.7; D-glucose, 10. It was pregassed with 95% O_2/5% CO_2. Slices were kept in a static condition for 25 min with the medium level at their upper surface. They were then perfused (surface covered) with gassed medium at a rate of 1 chamber volume/min.

Bipolar stimulating electrodes were positioned into the stratum radiatum and into the alveus in order to activate the Schaffer-commissural afferents of CA1 pyramidal cells and the axons of these cells. Micropipettes filled with 2 M NaCl (1 to 5 megohms impedance) were positioned into the apical dendritic zone of CA1 pyramidal cells to record the synaptic dendritic potentials and into the cell body layer of CA1 to record antidromic responses. Electrical stimulation consisted of 0.1-msec pulses of 4 to 10 V delivered at a frequency of 0.05 Hz. Drug solutions were adjusted to pH 7.4 with concentrated NaOH and were added to the perfusion medium for 2 to 5 min in volumes that constituted less than 10% of the perfusion volume. Drug perfusion was started after stable evoked responses were observed for a period of at least 10 min. Evoked potentials were quantified by measuring the amplitude of the negative component of the antidromic response and the slope of the first 600 μsec of the negative going component of the synaptic dendritic potential. Response analyses were performed on a MINC 11 computer (Digital Corp.).

Chemicals

L- and D-glutamic, L-aspartic, *N*-methyl D,L-aspartic, D,L-homocysteic, L-cysteine sulfinic, quisqualic, quinolinic, kainic, and D,L-α-aminoadipic acids were purchased from Sigma (St. Louis, MO). D,L-Aminophosphonobutyric acid was purchased from Calbiochem (La Jolla, CA).

Results

Desensitization to various agonists

Figure 1 illustrates the method that was used to quantify and compare the effects of amino acids. The sum of the percentages of decrease in the slope of the dendritic potential or in the amplitude of the negative component of the antidromic response was calculated for each drug, from the time at which the responses began to decrease until the time of complete recovery. The effect of the indicated amino acid was dose and time dependent. We assume that the reduction in field potential is due to the known depolarizing action of the tested amino acids.

A first 5-min application of L-glutamate (1 mM) induced a marked decrease of both the antidromic potential and the dendritic response (Fig. 1); the threshold concentration was between 0.1 and 0.5 mM. Full recovery of both potentials was observed by 6 to 8 min after the end of the drug application. However, successive perfusions for 5-min periods with the same concentration of L-glutamate were less and less effective. Thus, as is shown in Table I, the effect of L-glutamate, measured on the slope of the dendritic potential evoked by a Schaffer-commissural afferent volley, was reduced 7-fold after the third application. Similar results were obtained using the negative component of the antidromic response.

In several cases we noted that if a low concentration of L-glutamate was perfused for more than 5 min, the initial depolarization induced by the drug was not maintained but was reduced to a lower level. Such prolonged applications of excitatory amino acids were not systematically studied.

An apparent desensitization of the response to brief and successive applications was also found with D-glutamate, L-aspartate, L-cysteine sulfinate, and *N*-methyl-D,L-aspartate (Table I and Fig. 2, *A* and *B*). If a 30- to 40-min period was interposed between a third and a fourth application of any of these amino acids, then the drug-induced depolarization was nearly as great as that seen after the first exposure to the amino acid, indicating

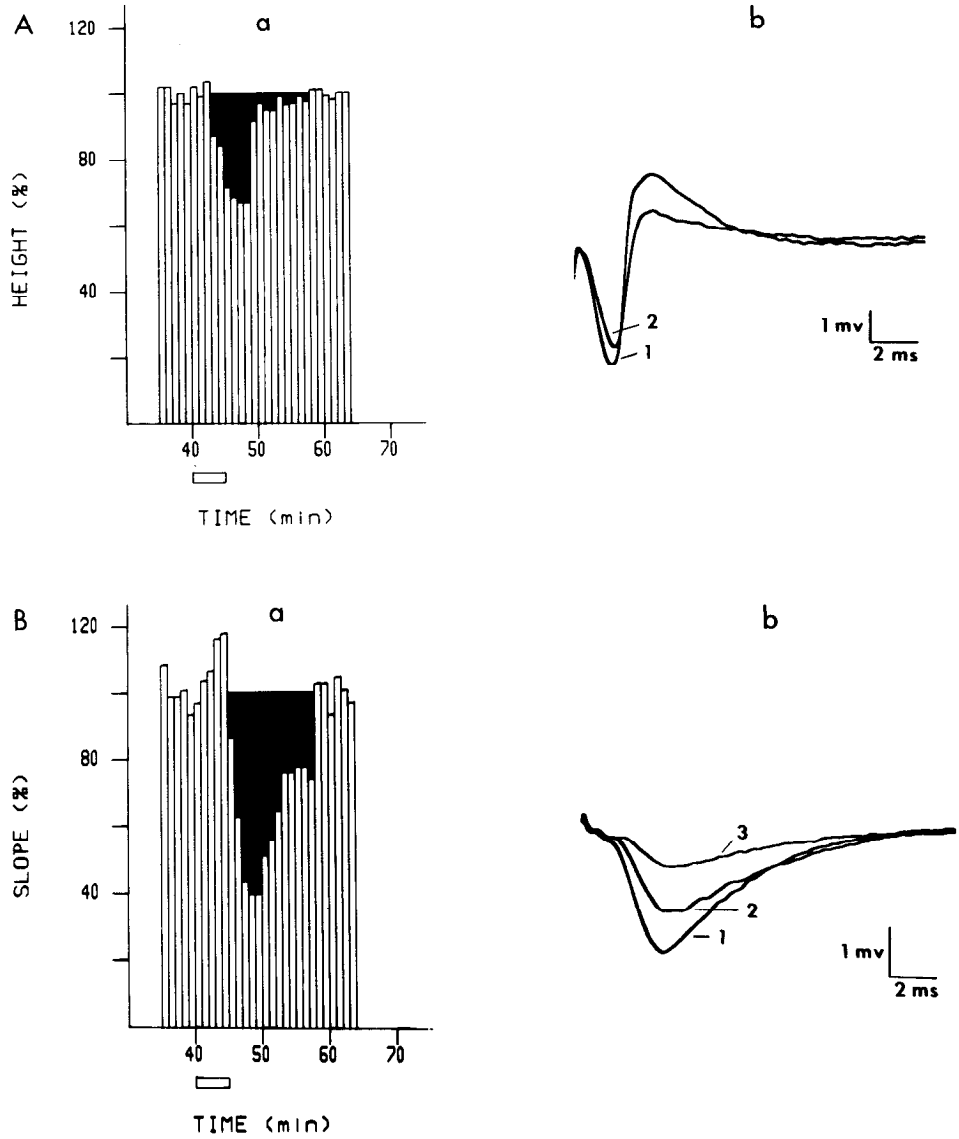

Figure 1. Quantification of the excitatory effect of acidic amino acids: Effect of 1 mM L-glutamate. *Aa*, The amplitudes of the negative component of the antidromic responses recorded before the first perfusion of the studied drug (1 mM L-glutamate: *open bar*) were measured, and their mean value during the predrug period was taken as the control value. The value of each response was then calculated for every minute as percentage of this control value. The effect of the tested drug was quantified by calculating the sum of the percentages of the decrease in the height of the response from the beginning of the effect until the full recovery of the response. This sum is represented on the histogram by the *dark area*. *Ab*, Antidromic response recorded in the cell body layer of CA1, in the absence of drug (*1*) and following a 5-min perfusion with 1 mM L-glutamate (*2*). *Ba*, The *black area* of the histogram represents an estimate of the effect of the tested amino acid (*open bar* = 1 mM L-glutamate) measured on the slope of the initial negative going potential of the synaptic response (see legend *Aa*). *Bb*, Synaptic dendritic potential recorded in the apical zone of CA1 pyramidal cells, in the absence of drug (*1*), following 3 min (*2*) and then 5 min (*3*) perfusion with 1 mM L-glutamate.

that the desensitization is reversible. It should be emphasized that neither the synaptic potential nor the antidromic response was reduced after successive applications of the amino acids at times when these agonists no longer elicited any significant effects (Fig. 2).

Brief applications of D,L-homocysteate also induced a rapid and pronounced decrease in the amplitude of the antidromic response and in the slope of the dendritic field potential. The threshold concentration of the effect was found to be 0.01 mM, and the maximal effect was obtained at a concentration of 0.10 to 0.20 mM. But, in contrast to effects of the amino acids described above, the response to a second and then to a third application of D,L-homocysteate was not significantly different from the response to a first perfusion of the drug (Table I and Fig. 2*D*). Similar results were obtained with kainate (Fig. 2*C*) and quisqualate. As shown in Figure 2, full recovery of the synaptic dendritic potential was obtained following

TABLE I

Effects of various excitatory amino acids on dendritic potentials during a first and a third application

The decrease in the slope of the dendritic potential during the perfusion of the various amino acids was quantified as described in the legend to Figure 1. Each value represents either the mean ± SEM or the individual values of the indicated number of experiments.

Amino Acids	Concentration/ Perfusion Period	Relative Potency		First Application	Third Application
			no.	*mean ± SEM*	*mean ± SEM*
L-Glutamate	1 mM/5 min	1	8	460 ± 86	57 ± 38
L-Aspartate	1 mM/5 min	1	4	454 ± 128	96 ± 23
L-Cysteine sulfinate	1 mM/5 min	0.25	2	163; 133	0; 12
D-Glutamate	1 mM/4 min	2	2	653; 694	249; 237
Quinolinate	0.10 mM/5 min	30	2	1123; 1614	NT[a]
N-Methyl-D,L-aspartate	0.01 mM/2 min	>200	4	468 ± 100	50 ± 31
D,L-Homocysteate	0.05 mM/5 min	20	3	474 ± 20	494 ± 79[b]
Kainate	0.01 mM/2 min	>400	2	597; 567	606; 771

[a] NT, not tested.

[b] Not significantly different from the value obtained following the first application (the Student's *t* test).

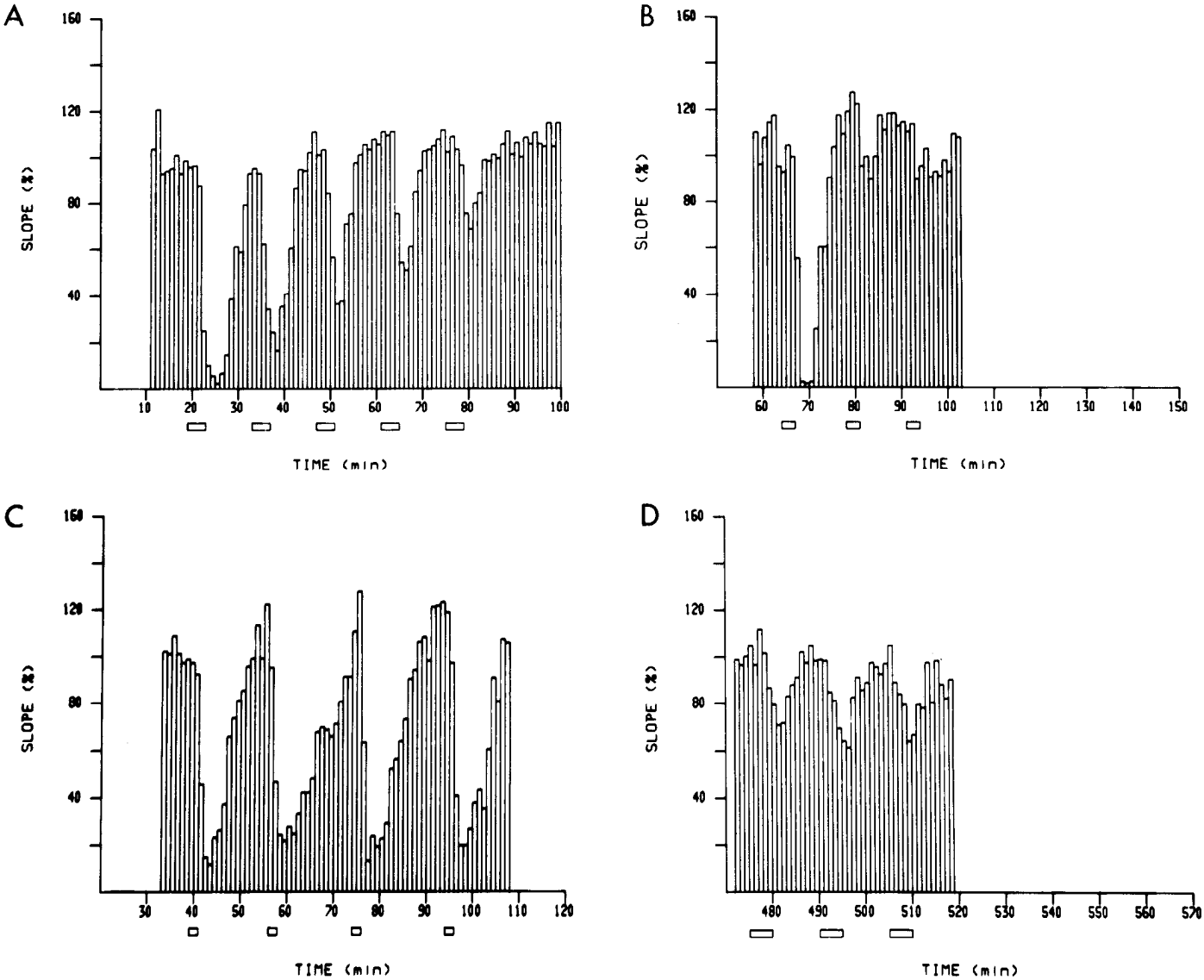

Figure 2. Examples of desensitizing and nondesensitizing responses to various acidic amino acids. The initial slope of the synaptic dendritic response was expressed as percentage of the control period (predrug) value as described in Figure 1. The histograms show the effect of successive applications of various agonists on this parameter. *A*, *Open horizontal bars*, 1 mM D-glutamate, 4-min period. *B*, 0.01 mM *N*-methyl-D,L-aspartate, 3-min period. *C*, 0.01 mM kainate, 2-min period. *D*, 0.05 mM D,L-homocysteate, 5-min period. The figure summarizes typical experiments which were replicated with similar results 4 times for *A*, 10 times for *B*, 5 times for *C*, and 20 times for *D*.

each repeated application of the amino acids. This was also observed for the antidromic response.

Cross-desensitization between various agonists

The effect of repeated applications of L-glutamate on the response to a second amino acid was tested and quantified using the procedure described in Figure 1. A normally effective dose of a second amino acid was applied 10 min after the last of three or four perfusions of L-glutamate. The physiological changes elicited by the test amino acid were compared either to the mean value of the responses to a first application measured in separate experiments (values reported in Table I) or to perfusion of the amino acid carried out 40 to 60 min before the first application of 1 mM L-glutamate (Fig. 3 and Table II).

Following desensitization to L-glutamate, the responses to L-aspartate (Fig. 3*A*) and D-glutamate showed a 90% depression compared to their respective normal values, whereas the effect of *N*-methyl-D,L-aspartate was reduced by 30 to 35%. In several cases, if more than 40 min was interposed between desensitization to L-glutamate and the perfusion of D-glutamate, L-aspartate, or *N*-methyl-D,L-aspartate, no significant change in the effect of these amino acids was observed. In marked contrast to these results, desensitization to L-glutamate apparently did not affect the response to D,L-homocysteate (Fig. 3*B*).

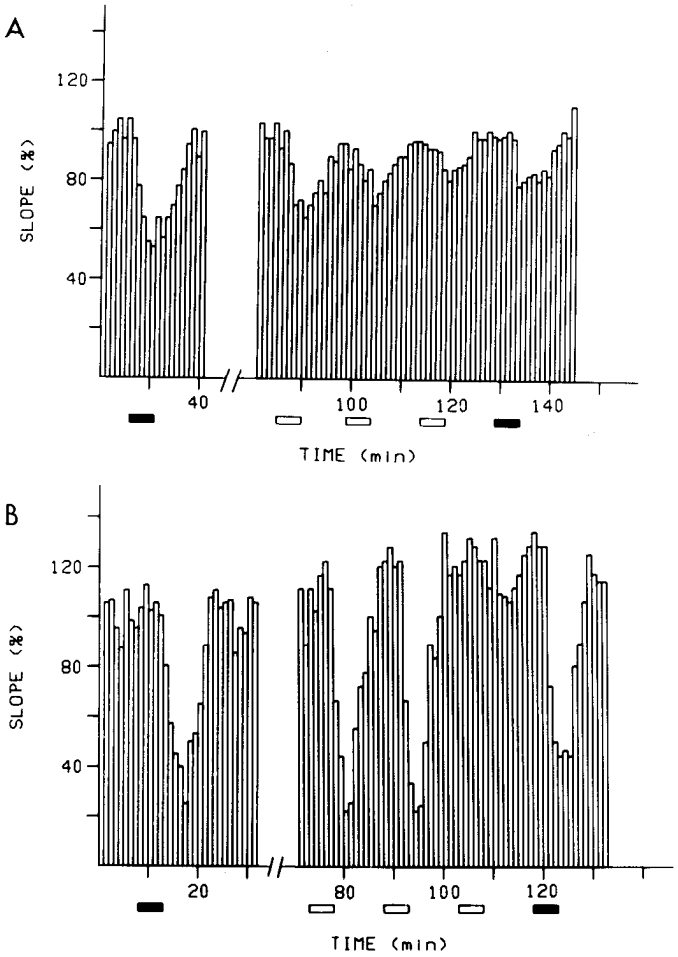

Figure 3. Cross-desensitization between the responses to various amino acids measured using the slope of the synaptic dendritic potential. *A*, The response to L-aspartate (1 mM, *solid bars*) was determined before and after three successive applications of L-glutamate (1 mM; *open bars*). *B*, The response to D,L-homocysteate (0.05 mM; *solid bars*) was determined before and after three successive applications of L-glutamate (1 mM, *open bars*). The results shown in *A* and *B* (single experiments) were similar to those obtained in 7 and 11 other experiments using the drug concentrations indicated on the figure.

TABLE II

Cross-desensitization between various amino acids

Following successive applications of a given amino acid (A_2), the response to a second amino acid (A_1) was quantified on the slope of the dendritic field potential using the procedure described in Figure 1. The first column summarizes the order of application of the tested amino acids. The second column gives the value (mean ± SEM of the indicated number of experiments (expressed as percentage of the value obtained following the first application) of the last response obtained after three or four successive perfusions of the drug A_2. The third column is the value of the response to the amino acid A_1 obtained 10 min after the last perfusion of A_2 expressed as percentage of the mean value of the responses to A_1 measured in separate experiments (values reported in Table I) or to a perfusion of A_1 performed 40 to 60 min before the first application of A_2.

$A_1/A_2/A_1$	Desensitization to A_2	Response to A_1 after Desensitization to A_2	No.
	% of control	*% of control*	
LA/LG/LA[a]	37 ± 9	46 ± 5	4
DG/LG/DG	25	10	1
NMDLA/LG/NMDLA	39; 36	71; 63	2
DLH/LG/DLH	31 ± 10	106 ± 14[b]	5
LG/LA/LG	25	30	1
LG/LCS/LG	8	33	1
LG/DG/LG	22	24	1
LG/NMDLA/LG	48; 7	6; 22	2
LG/DLH/LG	99 ± 7[b]	77 ± 22[b]	4

[a] LA, L-aspartate; LG, L-glutamate; DG, D-glutamate; NMDLA, *N*-methyl-D,L-aspartate; DLH, D,L-homocysteate; LCS, L-cysteine sulfinate.

[b] Not significantly different from the control value (the Student's *t* test).

Desensitization to L-aspartate or to L-cysteine sulfinate produced a 70% reduction in the response to L-glutamate. The L-glutamate response was also substantially reduced (74 to 94%) after repeated application of D-glutamate or *N*-methyl-D,L-aspartate. However, several successive brief applications of D,L-homocysteate did not significantly alter the physiological effects of a subsequent perfusion of L-glutamate (Table II).

It bears repeating that the occurrence of desensitization and cross-desensitization had no significant effect on synaptic field potentials.

Effects of various antagonists on synaptic transmission and exogenously applied acidic amino acids

In an effort to further discriminate synaptic from nonsynaptic actions of perfused amino acids, we tested

for their physiological effects in the presence of α-aminoadipate (AA) and aminophosphonobutyrate (APB), which are known to block synaptic transmission in the hippocampus (Watkins and Evans, 1981, for a review). During the period in which transmission was blocked, the effects of the various amino acids were tested using the antidromic response. Control data for a given amino acid were collected in two ways: (*1*) tests were carried out using the amino acid alone 40 min prior to perfusion with AA or APB (Fig. 4) and (*2*) the percentages of change produced by the amino acid alone were averaged from several experiments using different slices.

A continuous perfusion of 6 mM AA completely blocked the synaptic response, while inducing less than a 10% decrease in the amplitude of the negative component of the antidromic response. The effects appeared 5 to 10 min after the beginning of the perfusion and were stable throughout drug application. AA did not affect the response to a 1 mM, 5-min application of L-cysteine sulfinate nor to a 0.01 mM, 2-min application of kainate. The same dose of AA did not induce any significant change in the response to L-glutamate (5 mM, 5 min) but totally blocked the effect of *N*-methyl-D,L-aspartate (0.02 mM, 2 min) and produced a 60% reduction in the

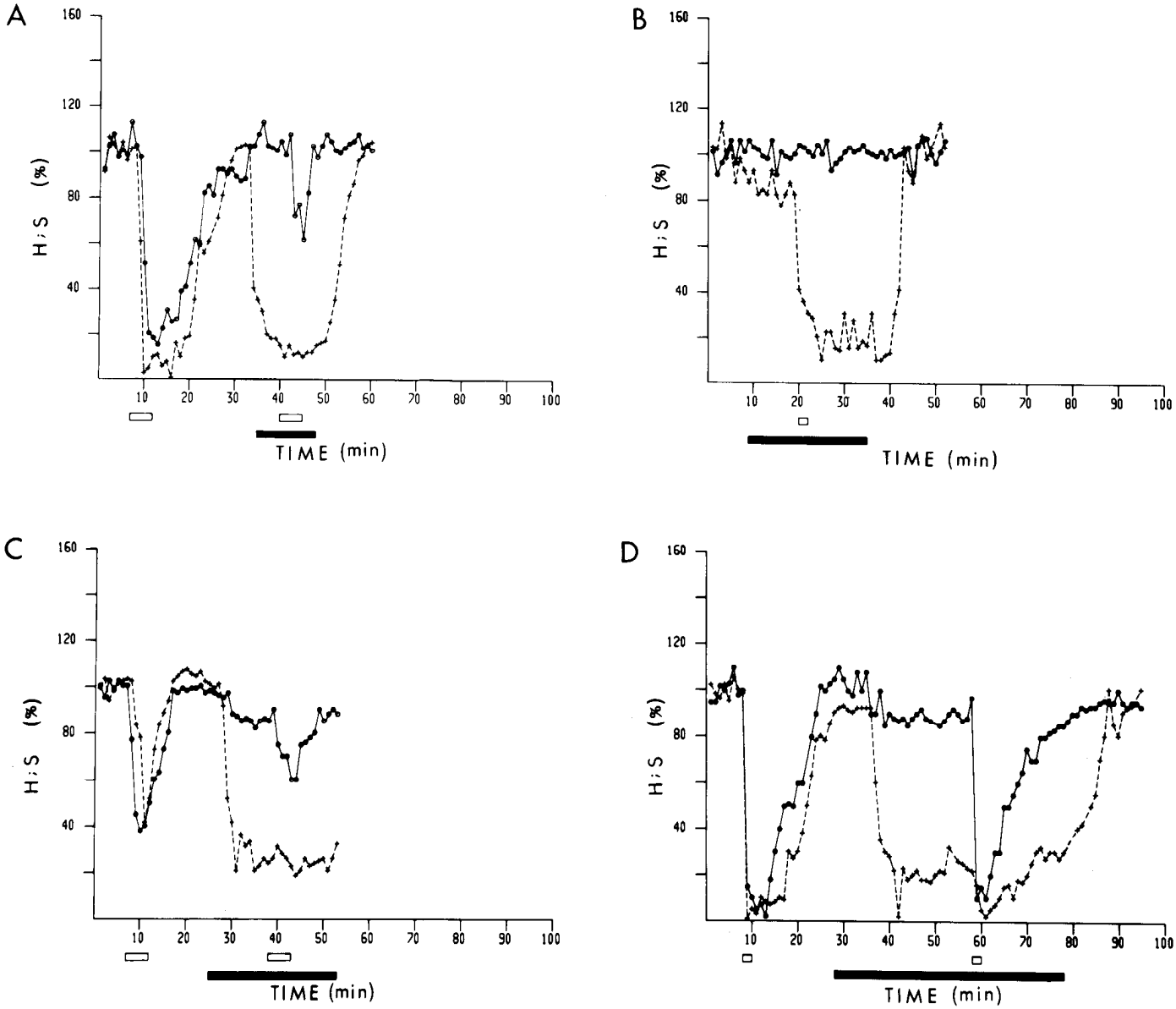

Figure 4. Effect of D,L-α-aminoadipate and D,L-aminophosphonobutyrate on the synaptic and antidromic responses of CA1 pyramidal cells. Each graph was calculated using the procedure described in Figure 1. *H* (*circle, solid line*), height of the negative component of the antidromic response. *S* (*crosses, dotted line*), slope of the initial negative going potential of the synaptic dendritic response. *A*, *open bars*, perfusion of D,L-homocysteate (DLH) (0.05 mM, 5 min), *solid bar*, perfusion of 6 mM D,L-α-aminoadipate (αAA). *B*, *open bar*, perfusion of *N*-methyl-D,L-aspartate (NMDLA) (0.02 mM, 2 min); *solid bar*, perfusion of 6 mM D,L-α-aminoadipate. *C*, *open bars*, perfusion of D,L-homocysteate (0.05 mM, 5 min); *solid bar*, perfusion of 5 mM D,L-aminophosphonobutyrate (APB). *D*, *open bars*, perfusion of *N*-methyl-D,L-aspartate (0.02 mM, 2 min); *solid bar*, perfusion of 5 mM D,L-aminophosphonobutyrate. The figure shows the results of typical experiments which were replicated with similar results four times (DLH/αAA), two times (NMDLA/αAA), four times (DLH/APB), and two times (NMDLA/APB).

response to D,L-homocysteate (0.02 to 0.05 mM, 5 min) (Fig. 4, *A* and *B*, Table III). In most of the experiments, a full recovery of the synaptic dendritic potential was observed 10 to 15 min after the end of the perfusion of 6 mM AA. In some cases, involving longer applications of AA, only a partial recovery of the synaptic potential was obtained.

Aminophosphonobutyrate produced an 80 to 90% decrease in the slope of the synaptic dendritic potential, which persisted during a prolonged infusion of the drug. This marked blocking effect on the synaptic transmission was accompanied by a 10 to 20% decrease in the amplitude of the negative component of the antidromic response. This is probably due to a weak agonist effect of the drug. In the presence of APB, the responses to L-cysteine sulfinate (1 mM, 5 min), L-glutamate (1 mM, 5 min), *N*-methyl-D,L-aspartate (0.02 mM, 2 min) (Fig. 4*D*), and kainate (0.01 mM, 2 min) were not significantly altered (Table IV). On the other hand, the same dose of APB produced a significant decrease in the response to D,L-homocysteate (0.02 to 0.05 mM, 5 min) (Fig. 4*C*, Table IV). The synaptic responses typically recovered following termination of 15 to 20 min of APB perfusion, but, as was the case for AA, a longer perfusion period occasionally produced an irreversible partial depression.

Discussion

The present results suggest that the multiple receptors for acidic amino acids in the hippocampus should be grouped into four categories (Tables V and VI). A first classification is obtained by considering the amino acids showing apparent desensitization; these include L- and D-glutamate, L-aspartate, L-cysteine sulfinate, and *N*-methyl aspartate. The fact that amino acids which are not substrates of the high affinity uptake system, such as D-glutamate or *N*-methyl aspartate (Balcar and Johnston, 1972), exhibit desensitization argues against the possibility that the effect results from a modified uptake of the compounds. A similar reasoning applies for the low affinity system because some substrates of this uptake process (e.g., D-glutamate) desensitize, whereas others (e.g., D,L-homocysteate) (Cox et al., 1977) do not. A reasonable explanation for the observed desensitization is a modification of the receptors similar to that reported for glutamate receptors at the crayfish neuromuscular junction (Takeuchi and Takeuchi, 1964) and in other invertebrate preparations (Nistri and Constantini, 1979) or the acetylcholine receptors in various systems (Katz and Thesleff, 1957). It is possible that the ionic channels regulated by these amino acids are shared between several agonists and are the sites of the desensitization. The partial cross-desensitization occurring between agonists for AA-sensitive and -insensitive receptors supports this idea. A similar desensitization occurring at the level of an ionic channel has been previously found for various transmitters in the parotid gland (Putney, 1977).

A second classification is provided by the differential blockade of the effects of amino acids by AA and APB. Although these antagonists are not completely selective, they partially discriminate between the amino acids used in the present experiments. Thus AA blocks the responses to *N*-methyl-D,L-aspartate, D-glutamate, and D,L-homocysteate but not to L-glutamate, L-cysteine sulfinate, or kainate. On the other hand, APB does not block the response to L-glutamate, L-cysteine sulfinate, *N*-methyl-D,L-aspartate, or kainate but inhibits the effects of D,L-homocysteate. This pattern is very similar to that obtained in a number of preparations (Collingridge and Davies, 1979; Hicks and McLennan, 1979; White et al., 1979; Davies and Watkins, 1982; Kemp and Sillito, 1982).

Combining these two classifications results in the definition of four types of receptors: (*1*) an NMA receptor

TABLE III

Effect of various amino acids on the amplitude of the antidromic response during continuous perfusion of 6 mM D,L-α-amino adipate

D,L-α-Aminoadipate was perfused until the synaptic response disappeared. Then, the D,L-α-aminoadipate perfusion was maintained, and the indicated concentrations of amino acids were perfused into the bath. The inhibition of the responses to the excitatory amino acids induced by D,L-α-aminoadipate was quantified by measuring the decrease in the amplitude of the antidromic response as described in the legend to Figure 1. Results are expressed as percentage of control values and represent the mean ± SEM or the individual values of the indicated number of experiments.

Amino Acids	Concentration/ Perfusion Period	% of Control	No.
L-Glutamate	5 mM/5 min	84 ± 5[a]	3
L-Cysteine sulfinate	1 mM/5 min	100, 100	2
N-methyl-D,L-aspartate	0.02 mM/2 min	0 ± 1[b]	3
D,L-Homocysteate	0.05–0.20 mM/5 min	40 ± 7[b]	5
Kainate	0.01 mM/2 min	100, 100	2
D-Glutamate	4 mM/5 min	29	1
Synaptic response to the Schaffer-commissural stimulation		0	12

[a] Not significantly different from the control value (the Student's *t* test).

[b] $p < 0.001$.

TABLE IV

Effect of various amino acids on the amplitude of the antidromic response during continuous perfusion of 5 mM D,L-aminophosphonobutyrate

D,L-Aminophosphonobutyrate (APB) was perfused until the synaptic response disappeared. Then, the APB perfusion was maintained, and the indicated concentrations of amino acids were perfused into the bath. The inhibition of the responses to the excitatory amino acids induced by APB was quantified by measuring the decrease in the amplitude of the antidromic response as described in the legend to Figure 1. Results are expressed as percentage of control values and represent the mean ± SEM or the individual values of the indicated number of experiments.

Amino Acids	Dose/Perfusion Period	% of Control	No.
L-Glutamate	5 mM/5 min	100,100	2
L-Cysteine sulfinate	1 mM/5 min	100,100	2
N-Methyl-D,L-aspartate	0.02 mM/2 min	100 ± 1	3
D,L-Homocysteate	0.05–0.20 mM/5 min	30 ± 19[a]	5
Kainate	0.01 mM/2 min	100,100	2
Synaptic response to the Schaffer-commissural stimulation		15 ± 5[a]	11

[a] $p < 0.001$.

TABLE V

D,L-α-Aminoadipate pharmacology: a comparison between desensitizable and nondesensitizable sites

	Desensitizable Sites	Nondesensitizable Sites
Not blocked by D,L-α-aminoadipate	L-Glutamate L-Cysteine sulfinate	Kainate
Blocked by D,L-α-aminoadipate	*N*-Methyl-D,L-aspartate D-Glutamate	D,L-Homocysteate Endogenous transmitter

TABLE VI

D,L-Aminophosphonobutyrate pharmacology: A comparison between desensitizable and nondesensitizable sites

	Desensitizable Sites	Nondesensitizable Sites
Not blocked by D,L-aminophosphonobutyrate	L-Glutamate L-Cysteine sulfinate *N*-methyl-D,L-aspartate	Kainate
Blocked by D,L-aminophosphonobutyrate		D,L-Homocysteate Endogenous neurotransmitter

activated by *N*-methyl aspartate and by D-glutamate which desensitizes and is blocked by AA but not by APB, (*2*) a kainate receptor which does not desensitize and is not blocked by AA or APB, (*3*) a L-glutamate/aspartate receptor which desensitizes and is not blocked by AA or APB (tentatively defined as a G_2 receptor), and (*4*) a receptor which does not desensitize, is activated by D,L-homocysteate, and is blocked by AA and APB (tentatively defined as a G_1 receptor).

This classification resembles that proposed by Watkins and Evans (1981) for spinal cord (except that we subdivide their quisqualate class into two groups) and the schema arrived at by Luini et al. (1981) from studies of amino acid-induced ^{22}Na fluxes in striatal slices.

Since the synaptic receptor does not appear to desensitize following exogenous application of amino acids, it is logical to assume that all the sites which exhibit desensitization represent extrasynaptic receptors. Therefore, the NMA receptor and the G_2 receptor are probably extrasynaptic. Because the kainate receptor is not blocked by AA or APB, whereas synaptic transmission is, it is likely that the kainate also stimulates an extrasynaptic receptor. This conclusion has also been reached based on studies of kainate's actions in the neonatal rat dorsal root (Davies and Watkins, 1979). Unlike the receptors for the other tested amino acids, the receptor stimulated by homocysteic acid shares a number of properties with the synaptic receptor for the Schaffer-commissural system. It is blocked by both APB and AA, as is synaptic transmission. Moreover, the homocysteic acid response is virtually unaffected by desensitization to several amino acids, and this is also true for synaptic potentials. The receptor for homocysteate is unusual in that it does not exhibit any evidence of desensitization; tests of ^{22}Na fluxes elicited by potassium-induced transmitter release suggest that the postsynaptic receptor also does not change with repeated episodes of stimulation (M. Baudry and G. Lynch, unpublished data).

The above arguments are based on studies of the Schaffer-commissural pathway and may not apply to the receptors for other hippocampal pathways.

The proposed classification includes the minimal number of receptors needed to account for the experimental results; there is a possibility that some of the categories are composites and that further division will be required. Cysteine sulfinate, for example, has been proposed to stimulate a receptor which is distinct from those for glutamate and aspartate (Recasens et al., 1982).

The present findings raise questions for the widely held hypothesis that aspartate and/or glutamate are transmitters in the Schaffer-commissural pathway. As shown by the present study, the physiological responses elicited by exogenously applied glutamate and aspartate do not have pharmacological sensitivities which accord with those of the synaptic transmitter. Thus these compounds do not meet the "identity of action" criterion required for a neurotransmitter. Despite this, we do not feel that it is warranted to conclude that neither of the amino acids is a neurotransmitter in the Schaffer-commissural systems. It is conceivable that the uptake systems prevent the concentration of perfused L-glutamate in the synaptic cleft from reaching levels high enough to stimulate the synaptic receptors, thereby restricting glutamate's effects to a population of extrasynaptic sites with properties distinct from those of the synaptic receptors. A similar hypothesis has been proposed previously on the basis of autoradiographic data (Hosli and Hosli, 1978), and it has been shown that uptake inhibitors potentiate the effect of glutamate on cat spinal neurons (Johnston et al., 1980).

In conclusion, the present study defines four types of excitatory amino acid receptors as well and suggests means for studying their participation in synaptic function. This classification may prove to be useful in analyzing complex neurophysiological phenomena such as epilepsy, and long-term potentiation, which are thought to involve alterations in excitatory amino acid receptors (Lynch and Baudry, 1983, for a review).

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