MOLECULAR PHARMACOLOGY, 24:229-234

Classification and Properties of Acidic Amino Acid Receptors in Hippocampus

III. Supersensitivity during the Postnatal Period and following Denervation

MICHEL BAUDRY, KATHRYN KRAMER, AND GARY LYNCH
Department of Psychobiology, University of California, Irvine, California 92717

Received December 15, 1982; Accepted May 4, 1983

SUMMARY

The effects of excitatory amino acids on ^{22}Na efflux rate in rat hippocampal slices were determined at various postnatal days and following removal of a major afferent system. Two weeks after a unilateral hippocampal aspiration, the ^{22}Na efflux induced by potassium ions, D-glutamate, *N*-methylaspartate, and kainate is significantly decreased in the contralateral intact hippocampus whereas the effect of L-glutamate is substantially increased. Analysis of concentration-response curves suggests that the increased responsiveness to L-glutamate is due to an increase in the maximal effect rather than to changes in the half-maximal concentration for the amino acid. Partial denervation does not detectably change efflux elicited by D,L-homocysteic acid nor does it modify the properties of [^3H]glutamate binding to hippocampal membranes. The effects of potassium ions, *N*-methylaspartate, and kainate but not of D,L-homocysteate are significantly decreased in slices incubated in the absence of calcium. All of the amino acids tested are considerably more potent in slices prepared from 11-day-old rats than in those from adult rats; the differences in responsiveness reflect an increase in maximal effect without changes in the half-maximal concentration. The responses to L-glutamate and D,L-homocysteate decline steadily between postnatal days 11 and 30, at which time adult values are reached. Together, the results from the denervation and development studies suggest a different localization and different modes of regulation for various classes of excitatory amino acid receptors.

INTRODUCTION

Intensive investigation over the past several years has revealed a considerable heterogeneity of receptors for the majority of neurotransmitters. Following the classification of muscarinic and nicotinic receptors for acetylcholine, it has been demonstrated that noradrenergic (1, 2), dopaminergic (3), serotonergic (4), and γ-aminobutyric acid (5) as well as opiate receptors (6) can be subdivided into various categories, mainly on the basis of different pharmacological specificities. Whether the different categories of receptors thus defined represent different molecular entities or the same proteins associated with different membrane elements remains unresolved (7). In some cases different receptors for the same transmitter are differentially localized or subject to different modes of regulation. For instance, it has been proposed that the dopamine D-3 receptor is located on the terminals of the dopaminergic neurons (8). Similarly, some *alpha*-adrenergic receptors are thought to be associated with the presynaptic terminals and have been implicated in the regulation of transmitter release (9). In addition, it has been demonstrated in various systems that the cellular distribution of transmitter receptors can be manipulated by several experimental conditions. Thus, although cholinergic receptors are uniformly distributed on the surface of muscle fibers before the establishment of neuromuscular junctions, they become highly concentrated in the postsynaptic site of the mature junction (10). Conversely, denervation results in the spreading of the receptors to the whole muscle surface accompanied by an increased rate of receptor synthesis and degradation (11).

In the previous paper, we characterized different classes of excitatory amino acid receptors in the hippocampus by using the ^{22}Na efflux assay in hippocampal slices (12). Using several criteria we defined a G_1 receptor (thought to be a transmitter receptor), an extrasynaptic glutamate receptor (G_2), an *N*-methylaspartate receptor, and a kainate receptor. The present paper is concerned with the possibility that these amino acid receptors are differentially localized or are subjected to different regulatory processes. Accordingly, we analyzed their prop-

This work was supported by National Science Foundation Grant BNS 81-12156-01 (to M. B.) and AFOSR Grant 82-01116 (to G. L.).

0026-895X/83/050229-06$02.00/0

erties under two different experimental conditions: (a) following removal of a massive afferent system that very likely uses an acidic amino acid as a transmitter (13), and (b) during the postnatal period in which most hippocampal synapses are established (14). As in the previous paper, we compared the properties of the receptors with those of the high-affinity membrane binding site for [^{3}H] glutamate (15).

MATERIALS AND METHODS

Sprague-Dawley rats were used in all of the studies. ^{22}Na (143 Ci/g; 200 μCi/ml) was purchased from the Radiochemical Center, Amersham (Arlington Heights, Ill.), and [^{3}H]glutamate (33 Ci/mmole) from ICN (Irvine, Calif.).

Measurement of ^{22}Na efflux in hippocampal slices. The stimulation of ^{22}Na efflux induced by various excitatory amino acids in hippocampal slices was measured using a method adapted from the procedure of Luini *et al.* (16) and Teichberg *et al.* (17) and described in the preceding paper (12). Briefly stated, rats were killed by decapitation, their brains were rapidly removed, and the hippocampi were dissected. Transverse sections (about 400 μm thick) were cut with a McIllwain tissue chopper and preincubated at 33° in an oxygenated physiological solution 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 24 tubes, each containing 1.0 ml of nonradioactive physiological solution saturated with O_2, at room temperature. Agonists were applied for 4 min, 17–20 min after loading. The results were expressed in terms of a specific efflux rate, and the effect of amino acids on this rate was calculated in terms of an index, *I*, as discussed in the preceding paper (12).

[^{3}H]Glutamate binding to hippocampal membranes. Hippocampal membranes were prepared and immediately assayed for [^{3}H]glutamate binding as described previously (15, 18). Proteins were determined by using the Coomassie blue dye technique devised by Bradford (19) as previously described (20).

Unilateral hippocampal aspiration. Removal of the commissural afferents to the hippocampal formation was accomplished by the aspiration of the contralateral hippocampal formation (with special care taken not to damage the septum ipsilateral to the lesion), with the rat under anesthesia induced by a mixture of ketamine and xylazine (1 mg–5 mg/100 g of body weight).

RESULTS

Effect of unilateral hippocampal aspiration on amino acid receptors. Two weeks following a unilateral hippocampal aspiration, the effect of excitatory amino acids on the ^{22}Na efflux rate was determined on slices prepared from the contralateral hippocampus and compared with that found in slices from control unoperated rats (Table 1). Whereas the efflux elicited by L-glutamate was increased by 40% in denervated slices, the effects of D-glutamate, *N*-methylaspartate, and kainate were decreased between 25% and 38%. The response of the slice to D,L-homocysteate was not affected by the lesion, whereas the effect of a depolarizing concentration of potassium ions is decreased by 40%. To establish whether the greater efficacy of L-glutamate was due to an increase in the apparent affinity of L-glutamate for its receptor or an increase in the maximal effect, we measured the amino acid-induced stimulation of ^{22}Na efflux rate across a range of concentrations (Fig. 1). L-Glutamate was significantly more effective at every tested concentration; the half-maximal concentration was 1.2 mM in the control slices and 1.15 mM in the lesioned group, whereas the maximal effect was calculated to be 5.1 in the control animals and 7.6 in the denervated animals. In contrast, the efflux elicited by various concentrations of D,L-homocysteate was not different between slices prepared from lesioned and control rats (Fig. 2).

The properties of the sodium-independent [^{3}H]glutamate binding sites in membranes prepared from the denervated hippocampus were only slightly different from those of control rats (Table 2). Although the B_{max}

TABLE 1

Effect of commissural lesions on the stimulatory effect of various amino acid agonists on the ^{22}Na efflux rate in hippocampal slices

The stimulatory effects of various excitatory amino acids and of potassium ions on the ^{22}Na efflux rate were determined in hippocampal slices from control rats and from commissurectomized rats 2 weeks following the unilateral hippocampal aspiration. The stimulatory effect was quantified in terms of a stimulatory index as described under Materials and Methods. The results are means ± standard error of the mean of six to eight experiments. Significance was determined by Student's *t*-test.

Agonist (concentration)	Control	Lesion	% Change
L-Glutamate (5 mM)	4.75 ± 0.35	6.72 ± 0.24	+41%[a]
D-Glutamate (1 mM)	4.08 ± 0.42	2.60 ± 0.31	−36%[b]
N-Methylaspartate (50 μM)	6.26 ± 0.40	4.61 ± 0.26	−24%[b]
Kainate (100 μM)	5.03 ± 0.24	3.54 ± 0.29	−30%[b]
D,L Homocysteate (100 μM)	5.34 ± 0.29	4.99 ± 0.23	−7%[c]
K^+ (50 mM)	2.09 ± 0.14	1.27 ± 0.23	−39%[b]

[a] $p < 0.01$.
[b] $p < 0.05$.
[c] Not significant.

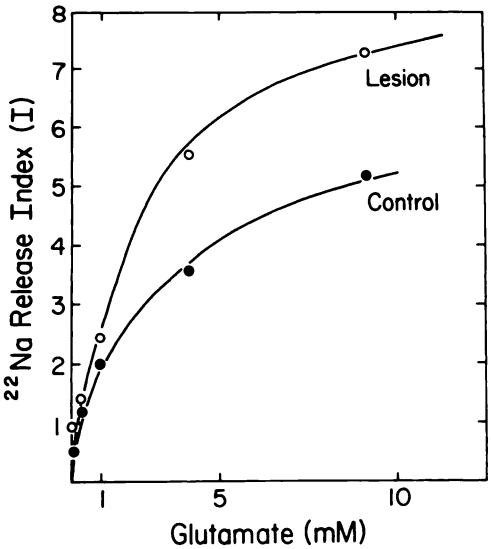

FIG. 1. *Effects of L-glutamate on the ^{22}Na efflux rate in hippocampal slices following commissural lesions*

^{22}Na efflux rate in hippocampal slices was determined as described under Materials and Methods 2 weeks following a unilateral hippocampal aspiration (*Lesion*) or in control rats (*Control*). The results are expressed in terms of the ^{22}Na release index (*I*) and are means of five experiments.

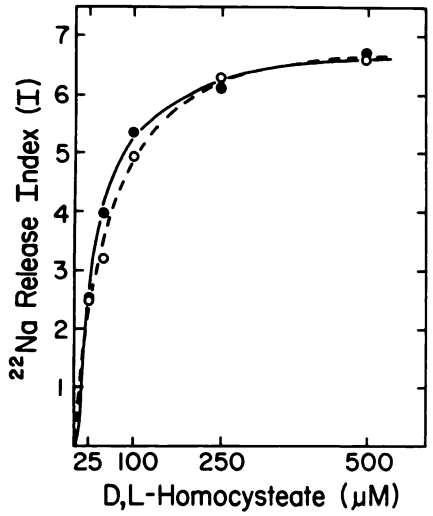

FIG. 2. *Effect of* D,L*-homocysteate on the* ^{22}*Na efflux rate in hippocampal slices following commissural lesions*

Same data as in legend to Fig. 1. ●, Lesion; ○, control.

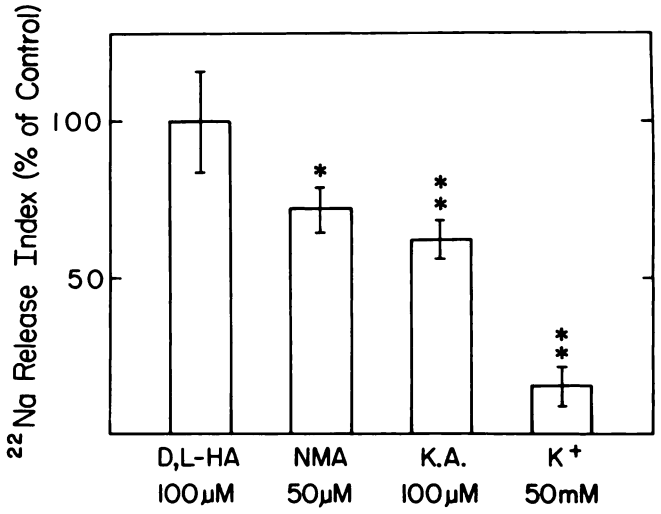

FIG. 3. *Effects of various agonists on the* ^{22}*Na efflux rate in hippocampal slices in the absence of calcium*

^{22}Na efflux rate in hippocampal slices was determined as described under Materials and Methods. Following the loading period in a normal incubation medium, slices were transferred for the efflux measure in normal medium or in a medium in which magnesium was substituted for calcium (calcium-free medium). The results were calculated in terms of the ^{22}Na release index and are expressed as ratios of the release index measured in the absence of calcium over the release index measured in the presence of calcium (percentage). Results are means ± standard error of the mean of six to eight experiments. * $p < 0.01$; ** $p < 0.001$ (Student's *t*-test). *D,L-HA*, D,L-homocysteate; *NMA*, *N*-methylaspartate; *K.A.*, kainate.

did not differ between the two groups, a small decrease in the K_d for [^{3}H]glutamate was observed in the lesioned animals.

Since the results of the lesion study pointed to a presynaptic action for potassium, *N*-methylaspartate, and kainate, we tested for stimulated efflux in slices incubated in the absence of calcium (Fig. 3). Under these conditions, the effect of potassium ions was decreased by almost 90%, and the effects of kainate and of *N*-methylaspartate were decreased by 30–40%; the action of D,L-homocysteate was not modified.

Characteristics of various glutamate receptors during postnatal development. The ^{22}Na efflux elicited by the amino acids was determined in slices prepared from 11-day-old animals and young adult (60 days old) rats (Table 3). All of the agonists tested were significantly more potent in the immature animals. This phenomenon was particularly marked for L-glutamate, which caused a 6-fold greater efflux in the neonatal rats than in the adults.

To determine whether the developmental differences were due to a change in affinity or in maximal effect, various concentrations of L-glutamate and D,L-homocysteate were applied to hippocampal slices prepared from 11-day-old rats or from adult rats (Fig. 4). For both amino acids, the age-related decline in potency clearly reflected a decrease in maximal effect without significant change in the half-maximal concentration. The pharmacological properties of the receptors appeared to be the same in the neonatal hippocampus as in the adult hippocampus (Fig. 5). Thus D-α-aminoadipate in the immature slices almost totally blocked the effects of D-glutamate, *N*-methylaspartate, and D,L-homocysteate; partially antagonized that of kainate; and did not significantly modify

TABLE 2

Effects of commissural lesions on the characteristics of [^{3}*H] glutamate binding to hippocampal membranes*

The characteristics of [^{3}H]glutamate binding to hippocampal membranes prepared from control rats and from commissurectomized rats were determined 2 weeks after a unilateral hippocampal aspiration. [^{3}H]glutamate binding was measured as previously described (15, 18), and the K_d and B_{max} were obtained by Scatchard analysis of the binding performed at a fixed [^{3}H]glutamate concentration and with increasing concentrations of cold glutamate. Numbers of experiments are indicated in parentheses. Results are means ± standard error of the mean. Significance was determined by Student's *t*-test.

	B_{max}	K_d
	pmoles/mg protein	*nM*
Control (3)	18.4 ± 0.1	710 ± 43
Lesion (3)	16.5 ± 0.8[a]	496 ± 39[b]

[a] Not significant.
[b] $p < 0.05$.

TABLE 3

Stimulatory effect of various agonists on ^{22}*Na efflux rates in slices from 11-day-old and adult rats*

The stimulatory effect of various excitatory amino acids on the ^{22}Na efflux rate was determined on hippocampal slices prepared from 11-day-old rats (PND 11) and 60-day-old rats (adult) as described under Materials and Methods. The results were calculated in terms of a ^{22}Na release index (*I*) as described; values are means ± standard error of the mean of four to six experiments.

Agonist	PND 11	Adult
L-Glutamate, 1 mM	13.5 ± 1.2	2.38 ± 0.12
D-Glutamate, 1 mM	13.8 ± 1.0	4.08 ± 0.62
N-Methylaspartate, 50 μM	10.4 ± 1.1	6.26 ± 0.40
Kainate, 100 μM	10.9 ± 1.2	5.03 ± 0.24
D,L-Homocysteate, 100 μM	13.6 ± 1.3	5.69 ± 0.11

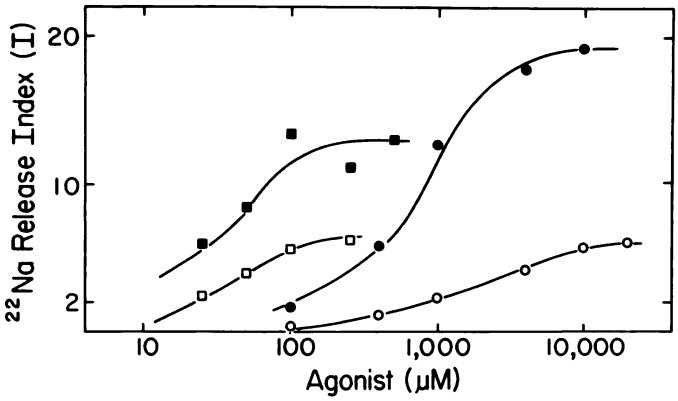

FIG. 4. *Effects of* L*-glutamate and* D,L*-homocysteate on the* ^{22}*Na efflux rate in hippocampal slices of 11-day-old or adult rats*

^{22}Na efflux rate in hippocampal slices was determined as described under Materials and Methods. The results were calculated in terms of the ^{22}Na release index and are the means of three or four experiments. □, ○, Adult rats (60 day-old); ■, ●, 11-day-old rats. ○, ●, L-glutamate. □, ■, D,L-homocysteate.

responses to L-glutamate. A similar pattern was previously found in the adult hippocampus [see figure 5 in the accompanying paper (12)].

The effects of a fixed concentration of L-glutamate and

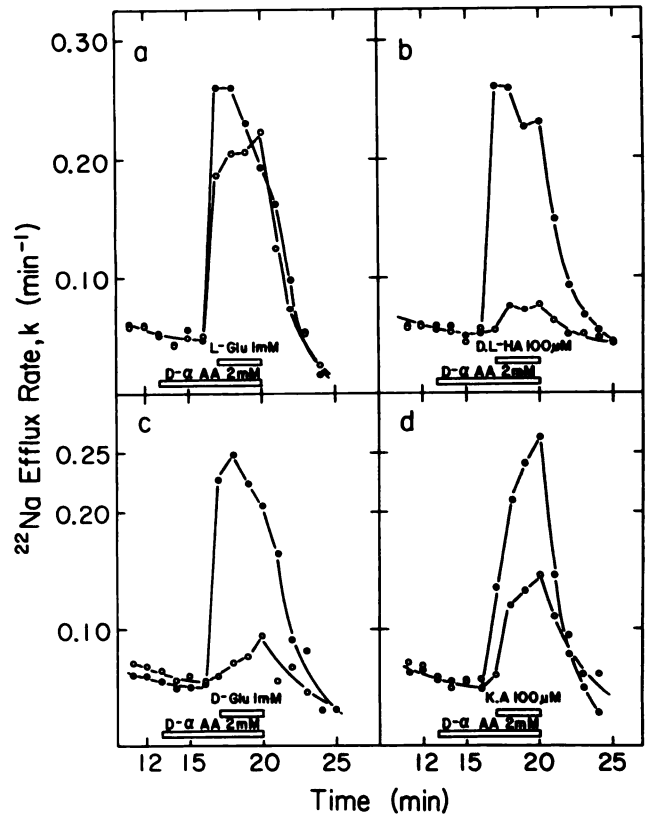

FIG. 5. *Effect of* D*-α-aminoadipate (D-α AA) on the stimulation of* ^{22}*Na efflux rate elicited by various excitatory amino acids in hippocampal slices from 11-day-old rats*

^{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 in terms of the specific ^{22}Na efflux rate, *k*, and are means of values from two experiments which differed by less than 10%. ●, Agonists alone; ○, in the presence of 2 mM D-α-aminoadipate.

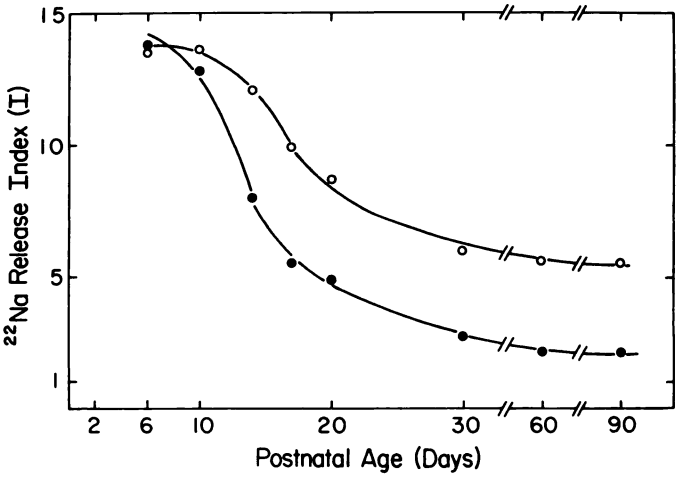

FIG. 6. *Effect of* L*-glutamate and* D,L*-homocysteate on the* ^{22}*Na efflux rate in hippocampal slices at various postnatal ages*

^{22}Na efflux rate in hippocampal slices was determined as described under Materials and Methods. L-Glutamate (1 mM, ●) and D,L-homocysteate (100 μM, ○) were applied for 4 min and their stimulatory effect on the ^{22}Na efflux rate was expressed in terms of the ^{22}Na release index. Each point represents the mean of at least two experiments which differed by less than 15%.

D,L-homocysteate on the ^{22}Na efflux rate were determined in hippocampal slices prepared from rats of various postnatal ages (Fig. 6). Both L-glutamate and D,L-homocysteate were maximally effective between postnatal days 6 and 10 and then progressively less so until day 30, at which time adult levels of elicited efflux were reached.

We previously described the postnatal development of [^{3}H]glutamate binding to hippocampal membranes (21). Briefly stated the total number of binding sites increased by a factor of 10 between postnatal days 4 and 10 and further increased by a factor of 2 between postnatal day 10 and adulthood. However, the density of binding sites (expressed as picomoles per milligram of protein) reached a maximum at postnatal day 10 and slowly decreased to reach the adult level by postnatal day 22.

DISCUSSION

The present results indicate that the four types of amino acid receptors we previously defined (12) are subjected to different modes of regulation and/or are located on different cellular elements. Removing the hippocampal commissural projections, which contribute about 40% of the nerve terminals in this structure (22), results in a 40% decrease in the efflux elicited by potassium ions. This fits well with the idea (16) that a high potassium concentration stimulates the ^{22}Na efflux rate by inducing the release of the endogenous transmitter(s) which in turn opens sodium channels. This treatment also results in a 50% increase in the maximal responsiveness of L-glutamate on the ^{22}Na efflux rate in hippocampal slices without significant change in its half-maximal concentration. This result is in agreement with a previous report showing that this lesion results in an increased depolarizing effect of iontophoretically applied L-glutamate (23). Several arguments suggest that the increased reponsiveness to L-glutamate represents a true supersensitivity of the denervated hippocampus to this agonist. First, al-

though it has been shown that commissural lesions results in a significant decrease in the high-affinity uptake of L-glutamate (24), a decrease in uptake is not likely to explain the increase in the maximal responsiveness to L-glutamate since it would simply shift to the left the dose-response curve for the amino acid. Second, a denervation-induced change in the cellular distribution of ^{22}Na ions is also an unlikely explanation since this would require the selective modification of a pool of sodium ions "mobilizable" by L-glutamate but not by any other excitatory amino acids (see ref. 12 for a discussion of this point). Finally, Luini *et al.* (16) showed that the effect of L-glutamate on the sodium efflux rate in striatal slices is not modified in the absence of calcium, suggesting that this effect is postsynaptic. Therefore we favor the idea that the greater responsiveness to L-glutamate is due to an increased number of L-glutamate receptors (G_2 receptors, according to our classification), similar to the mechanism reported for glutamate receptors in insect muscle (25) and for various neurotransmitter receptors following denervation or chronic blockade (26).

In marked contrast to the results obtained with L-glutamate, denervation caused a significant *decrease* in the ^{22}Na efflux stimulated by D-glutamate, *N*-methylaspartate, and kainate. This finding suggests that at least some receptors for these amino acids are located on the terminals removed by the lesion. In support of this suggestion, the effects of the three agonists are also significantly decreased when transmitter release is blocked by removal of calcium. The neurotoxic effect of kainic acid requires the presence of presynaptic excitatory synapses (27), and more recently kainic acid has been shown to induce a calcium-dependent release of glutamic and aspartic acids in hippocampal slices (28). Evidence of this type has led several authors to propose that kainic acid receptors are localized on nerve terminals. *N*-Methylaspartate and D-glutamate are likely to stimulate one class of receptors, and our results suggest that these are located in part on the presynaptic elements. The absence of effect of commissural lesions or of removal of calcium on the response to D,L-homocysteate strongly supports the idea that this amino acid preferentially stimulates a postsynaptic receptor. In this regard, it is of interest that the properties of the sodium-independent [^3H]glutamate binding sites in hippocampal membranes are also not modified by the commissural lesions. This result excludes a presynaptic localization of these sites. The small decrease in the apparent affinity of [^3H]glutamate for the binding site might be due to a decrease in the amount of glutamate in the deafferented hippocampus (24), since endogenous glutamate may contaminate the membrane preparation (29).

The apparent postsynaptic locus of both the sodium-independent [^3H]glutamate binding site and the homocysteic acid receptor raises the possibility that these two entities are related. Previous evidence has also pointed to this conclusion. Homocysteate has a high affinity for the binding site, and α-aminoadipate is a very effective antagonist of both the binding site and the homocysteic acid receptor (15, 30).

There is also reason to believe that the binding site/homocysteic acid receptor (G_1) is linked to the receptor for the endogenous neurotransmitter: (a) both the G_1 site and transmitter receptor are postsynaptic (see above); (b) both are blocked by α-aminoadipate; (c) both are linked to an increase in sodium conductance (12, 31); and (d) in marked contrast to the receptors for all other amino acids tested, both the G_1 and transmitter receptor do not exhibit desensitization (30).

Measurement of excitatory amino acid-induced ^{22}Na efflux in slices from rats of various postnatal ages provided complementary information on the modes of regulation of the various classes of excitatory amino acid receptors. All of the excitatory amino acids tested appeared to be more potent in immature rats than in adult rats. One possible explanation for such an effect could be a differential distribution of ^{22}Na ions in neonatal and adult tissues; for instance, since glial cells develop mainly after the first 2 postnatal weeks in hippocampus (14), it is conceivable that, in slices from younger rats, a greater percentage of the labeled sodium ions is located in neuronal elements and therefore releasable by excitatory agonists. However, this should result in a similar ratio in the potency of the various amino acids between neonatal and adult slices. This is clearly not the case. A ceiling effect is also not likely to explain the observed differences, since the release was far from the maximal values for several of the amino acids. Differences in amino acid uptake processes between neonatal and adult slices would be expected to shift dose-response curves to the left, but not to modify maximal responsiveness. Therefore the larger responsiveness to most of these amino acids probably represents a true supersensitivity. This agrees with previously reported marked supersensitivity to the effect of L-glutamate on the accumulation of cyclic GMP in neonatal cerebellar slices (32). We have also noted that the depolarizing effect of L-glutamate is more pronounced in neonatal hippocampal slices than in adult hippocampal slices.[1] Possibly then, neurons, like muscle cells, exhibit a wide distribution of neurotransmitter receptors on developing dendritic trees, and the formation of synapses results in the partial elimination of extrasynaptic receptors and the "focalization" of the transmitter receptors at the postsynaptic sites. The time course of the disappearance of the extrasynaptic glutamate G_2 receptors (between postnatal days 11 and 30) indeed coincides with the formation of the majority of synapses in the hippocampal formation (14).

The maximal response to D,L-homocysteate also decreases during the postnatal period. Interestingly, the density of [^3H]glutamate binding sites to hippocampal membranes (i.e., binding per unit membrane protein) decreases over postnatal development with a time course that parallels that for the decline of homocysteate-induced stimulation of ^{22}Na efflux, providing further support for the idea that the binding site is linked to the homocysteic acid receptor.

In conclusion, the presumed extrasynaptic G_2 glutamate receptors exhibit the phenomenon of supersensitivity during postnatal development and following removal of postulated glutamatergic pathways, but the synaptic G_1 glutamate receptors do not seem to obey the same regulatory mechanisms. In this way neurons resemble skeletal muscles, and studies comparing the cellular

[1] L. Fagni *et al.*, unpublished data.

processes underlying the supersensitivity phenomenon in the two systems will be of interest. More generally, the existence of extrasynaptic receptors in neurons in the central nervous system raises important questions concerning their potential functional role in synaptic transmission. As we mentioned previously (33) the fact that these receptors can be desensitized without affecting synaptic transmission suggests that they do not participate in the "normal" operation of the brain. However, they may play a role in some abnormal or pathological conditions such as epilepsy or in aged brains, where a decreased uptake of glutamate has been reported (34). In addition, the presynaptic localization of some of these extrasynaptic receptors suggests that they might be involved in the modulation of transmitter release.

ACKNOWLEDGMENTS

The authors wish to thank Andrej Rotter, of the Department of Pharmacology, University of California, Irvine, Medical School, for providing access to the γ-scintillation counter; Michael Berns, of the Department of Developmental and Cell Biology, for generously providing the rat litters used in this study; and Karen Zfaty for her secretarial assistance.

REFERENCES

1. Lands, A. M., A. Arnold, J. P. McAuliff, F. P. Luduena, and T. G. Brown. Differentiation of receptor systems by sympathomimetic amines. *Nature (Lond.)* **214**:597–598 (1967).
2. Lefkowitz, R. J. Identification and regulation of alpha- and beta-adrenergic receptors. *Fed. Proc.* **37**:123–129 (1978).
3. Seeman, P. Brain dopamine receptors. *Pharmacol. Rev.* **32**:229–313 (1980).
4. Nelson, D. L., A. Herbet, S. Bougoin, J. Glowinski, and M. Hamon. Characteristics of central 5-HT receptors and their adaptive changes following intracerebral 5,7-dihydroxytryptamine administration in the rat. *Mol. Pharmacol.* **14**:983–995 (1978).
5. Nistri, A., and A. V. Constantini. Pharmacological characterization of different types of GABA and glutamate receptors in vertebrates and invertebrates. *Prog. Neurobiol.* **13**:117–235 (1979).
6. Lord, J. A. H., A. A. Waterfield, J. Hughes, and H. W. Kosterlitz. Endogenous opioid peptides: multiple agonists and receptors. *Nature (Lond.)* **267**:495–500 (1977).
7. Leysen, J. E., and W. Gommeren. Optimal conditions for ^{3}H-apomorphine binding and anomolous equilibrium binding of ^{3}H-apomorphine and ^{3}H-spiperone to rat striatal membranes: involvement of surface phenomena versus multiple binding sites. *J. Neurochem.* **36**:201–219 (1981).
8. Sokoloff, P., M. P. Martres, and J. C. Schwartz. ^{3}H-Apomorphine labels both dopamine postsynaptic receptors and autoreceptors. *Nature (Lond.)* **288**:283–286 (1980).
9. Starke, K., H. D. Taube, and E. Borowski. Presynaptic receptor systems in catecholaminergic transmission. *Biochem. Pharmacol.* **25**:259–268 (1977).
10. Devreotes, P. N., and D. M. Fambrough. Acetylcholine receptor turnover in membranes of developing muscle fibers. *J. Cell Biol.* **65**:335–358 (1975).
11. Pumplin, D. W., and D. M. Fambrough. Turnover of acetylcholine receptors in skeletal muscle. *Annu. Rev. Physiol.* **44**:319–335 (1982).
12. Baudry, M., K. Kramer, L. Fagni, M. Recasens, and G. Lynch. Classification and properties of acidic amino acid receptors in hippocampus. II. Biochemical studies using the sodium efflux assay. *Mol. Pharmacol.* **24**:222–228 (1983).
13. Storm-Mathisen, J. Localization of transmitter candidates in the brain: the hippocampal formation as a model. *Prog. Neurobiol.* **8**:119–181 (1977).
14. Loy, R. Development of afferent lamination in Amnon's horn of the rat. *Anat. Embryol.* **159**:257–275 (1980).
15. Baudry, M., and G. Lynch. Characterization of two ^{3}H-glutamate binding sites in rat hippocampal membranes. *J. Neurochem.* **36**:811–820 (1981).
16. Luini, A., D. Goldberg, and V. Teichberg. Distinct pharmacological properties of excitatory amino acid receptors in the rat striatum: study by the Na^+ efflux assay. *Proc. Natl. Acad. Sci. U. S. A.* **78**:3250–3254 (1981).
17. Teichberg, V. I., D. Goldberg, and A. Luini. The stimulation of ion fluxes in brain slices by glutamate and other excitatory amino acids. *Mol. Cell. Biochem.* **39**:281–295 (1981).
18. Baudry, M., and G. Lynch. Regulation of hippocampal glutamate receptors: evidence for the involvement of a calcium-activated protease. *Proc. Natl. Acad. Sci. U. S. A.* **77**:2298–2302 (1980).
19. Bradford, M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **72**:248–254 (1976).
20. Lynch, G., S. Halpain, and M. Baudry. Effects of high-frequency synaptic stimulation on glutamate receptor binding studied with a modified in vitro hippocampal slice preparation. *Brain Res.* **244**:101–111 (1982).
21. Baudry, M., D. Arst, M. Oliver, and G. Lynch. Development of glutamate binding sites and their regulation by calcium in rat hippocampus. *Brain Res.* **1**:37–48 (1981).
22. Goldowitz, D., S. W. Scheff, and C. W. Cotman. The specificity of reactive synaptogenesis: a comparative study in the adult rat hippocampal formation. *Brain Res.* **170**:427–441 (1979).
23. Segal, M. Supersensitivity of hippocampal neurons to acidic amino acids in decommissurized rats. *Brain Res.* **119**:476–479 (1977).
24. Storm-Mathisen, J. Glutamic acid and excitatory nerve endings: reduction of glutamic acid uptake after axotomy. *Brain Res.* **120**:379–386 (1977).
25. Gration, K. A. F., R. B. Clark, and P. N. R. Usherwood. Denervation of insect muscle: a comparative study of the changes in L-glutamate sensitivity on locust retractor unguis and extensor tibiae muscle. *Neuropharmacology* **18**:201–208 (1979).
26. Schwartz, J. C., J. Costentin, M. P. Martres, P. Protais, and M. Baudry. Modulation of receptor mechanisms in the CNS: hyper- and hyposensitivity to catecholamines. *Neuropharmacology* **17**:665–680 (1978).
27. Nadler, J. V., and G. J. Cuthbertson. Kainic and neurotoxicity towards hippocampus: dependence on specific excitatory pathways. *Brain Res.* **195**:45–56 (1980).
28. Ferkany, J. W., R. Zaczek, and J. T. Coyle. Kainic acid stimulates excitatory amino acid neurotransmitter release at presynaptic receptors. *Nature (Lond.)* **298**:757–759 (1982).
29. Sharif, N. A., and P. J. Roberts. Problems associated with the binding of L-glutamic acid to synaptic membranes: methodological aspects. *J. Neurochem.* **34**:779–784.
30. Fagni, L., M. Baudry, and G. Lynch. Classification and properties of excitatory amino acid receptors in hippocampus. I. Electrophysiological studies of an apparent desensitization and interactions with drugs which block transmission. *J. Neurosci.* (in press).
31. Hablitz, J. J., and I. A. Langmoen. Excitation of hippocampal pyramidal cells by glutamate in the guinea-pig and rat. *J. Physiol. (Lond.)* **325**:317–331 (1982).
32. Garthwaite, J., and R. Balazs. Supersensitivity to the cyclic GMP response to glutamate during cerebellar maturation. *Nature (Lond.)* **275**:328–330 (1978).
33. Fagni, L., M. Baudry, and G. Lynch. Desensitization to glutamate does not affect synaptic transmission in rat hippocampal slices. *Brain Res.* **261**:167–171 (1982).
34. Price, M. T., J. W. Olney, and R. Haft. Age-related changes in glutamate concentration and synaptosomal glutamate uptake in adult rat striatum. *Life Sci.* **28**:1365–1370 (1981).

Send reprint requests to: Dr. Michel Baudry, Department of Psychobiology, University of California, Irvine, Calif. 92717.