Facilitation of glutamate receptors enhances memory
(chronic recording/synaptic responses/spatial mazes/olfactory learning)

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Communicated by Richard F. Thompson, October 21, 1993

ABSTRACT A benzamide drug that crosses the blood-brain barrier and facilitates dL-α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor-mediated synaptic responses was tested for its effects on memory in three behavioral tasks. The compound reversibly increased the amplitude and prolonged the duration of field excitatory postsynaptic potentials in hippocampal slices and produced comparable effects in the dentate gyrus in situ after intraperitoneal injections. Rats injected with the drug 30 min prior to being given a suboptimal number of training trials in a two-odor discrimination task were more likely than controls to select the correct odor in a retention test carried out 96 hr later. Evidence for improved memory was also obtained in a water maze task in which rats were given only four trials to find a submerged platform in the presence of spatial cues; animals injected with the drug 30 min before the training session were significantly faster than vehicle-injected controls in returning to the platform location when tested 24 hr after training. Finally, the drug produced positive effects in a radial maze test of short-term memory. Well-trained rats were allowed to retrieve rewards from four arms of an eight-arm maze and then tested for reentry errors 8 hr later. The number of such errors was substantially reduced on days in which the animals were injected with the drug before initial learning. These results indicate that a drug that facilitates glutamatergic transmission enhances the encoding of memory across tasks involving different sensory cues and performance requirements. This may reflect an action on the cellular mechanisms responsible for producing synaptic changes since facilitation of AMPA receptors promotes the induction of the long-term potentiation effect.

Recent work indicates that facilitation of dL-α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) (glutamate) receptor-mediated transmission in slices of hippocampus reduces the amount of afferent stimulation needed to induce a maximal degree of long-term potentiation (LTP) without changing the LTP "ceiling" itself (1). Because there is evidence implicating LTP as a substrate for certain types of memory (2), it is possible that drugs that produce such effects in brain will reduce the amount of training needed for the formation of robust memory. The experiments reported here tested this idea by using a drug that crosses the blood-brain barrier and enhances synaptic responses in freely moving animals.

The carbonic anhydrase inhibitor cyclothiazide and the nootropic compound aniracetam enhance excitatory transmission in vitro by prolonging the open time of glutamate (AMPA) receptors (3-6). Cyclothiazide probably does not cross the blood-brain barrier (7) and aniracetam is rapidly metabolized in peripheral tissues to anisoyl γ-aminobutyric acid (GABA) (8), which we have found to have little effect on glutamatergic transmission. Accordingly, we synthesized a series of compounds that lack the labile imide function of aniracetam and tested their effects first with in vitro slices and then in brain after peripheral administration. Three separate structural modifications yielded a drug [1-(1,3-benzodioxol-5-ylcarbonyl)pyrperidine] with the requisite pharmacological characteristics. This compound was used in three behavioral paradigms and found to substantially improve memory encoding in each case. These results may be of significance for efforts to develop therapeutics directed at memory disorders.

MATERIALS AND METHODS

Slices of hippocampus were prepared and maintained in an interface chamber using conventional methods. Field excitatory postsynaptic potentials (EPSPs) were recorded in the stratum radiatum of region CA1b and elicited by single stimulation pulses delivered once per 20 sec to a bipolar electrode positioned in the Schaffer commissural projections. Measures of the slope, amplitude, and duration of the responses were collected from digitized records.

Chronic stimulation electrodes were implanted in the perforant path and chronic recording electrodes were placed in the hilus of deeply anesthetized male rats (Long-Evans; 250 g). The final positions of the electrodes were determined by physiological criteria; i.e., the production of a large, short-latency field EPSP with minimal stimulation currents. The electrodes were then attached to a head connector, which was permanently affixed to the skull. One week later the rats were acclimated to a recording cage and to the attachment of a recording lead to the head connector. Recordings were collected for 2–3 days before the start of experimental sessions to ensure the stability of the stimulation–recording arrangements (see ref. 9 for a more complete description). For drug testing, the rats were placed in the cage and 60–90 min of baseline recording was conducted; in most cases, the animals were then injected with vehicle (cyclo-dextrin) and then 30–45 min later with vehicle plus the experimental benzamide compound (120 mg/kg). Recordings were then continued for 2–3 hr. Seven rats were used, most of which were tested twice with the drug.

The behavioral equipment and paradigms used in the studies are described below; different groups of adult male rats were used in the separate experiments.

RESULTS

Fig. 1B and D illustrates results from slices for the benzamide; as shown, the compound produced an increase in the amplitude of the field EPSP and extended its duration and did so to a much greater extent than an equivalent concentration of aniracetam (Fig. 1A and C). A number of benzamide compounds have been synthesized and shown to be more

Abbreviations: AMPA, dL-α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; LTP, long-term potentiation; EPSP, excitatory postsynaptic potential; GABA, γ-aminobutyric acid.
potent than aniracetam but structure–activity relationships have not yet been established. Results from i.p. injections are summarized in Fig. 1E and F. Peripherally administered drug enhanced the field EPSP evoked by perforant path stimulation in freely moving rats (Fig. 1F) much in the way that it did for responses elicited in slices. The effect had a rapid onset and persisted for considerable periods as shown in Fig. 1E; injections of the carrier vehicle had no effects on the evoked responses. Similar results were obtained from seven rats with chronically implanted electrodes (Table 1). These findings indicate that the compound crosses the blood–brain barrier and produces a reliable facilitation of central synapses. Rapid transport to brain after systemic injections has also been observed with positron-emission tomography (PET) scans using C-11-labeled analogue and variants of the benzamide have been found to produce physiological effects similar to those shown in Fig. 1 in anesthetized rats (G.R., J. Larson, P. Xiao, and G.L., unpublished data). Aniracetam at concentrations 4 times greater than those used in the experiments described above (i.e., at concentrations approaching the limit of solubility) had no detectable effect on the field EPSP in three rats with chronically implanted electrodes.

Behavioral studies were carried out with i.p. injections at dosages found to enhance glutamatergic transmission. The
Table 1. Percentage increases for three synaptic response parameters after i.p. injections of a drug (AMPA) that modulates glutamate receptors

<table>
<thead>
<tr>
<th>Parameter</th>
<th>% increase, mean ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amplitude</td>
<td>19 ± 12*</td>
</tr>
<tr>
<td>Half-width</td>
<td>12 ± 2**</td>
</tr>
<tr>
<td>Area</td>
<td>32 ± 15**</td>
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</table>

Evoked responses elicited by stimulation pulses (3 per min) delivered to the perforant path were recorded in the hilus of dentate gyrus of freely moving rats. Average values for all responses collected for 30 min before and 30 min after injection of the drug (320 mg/kg) were compared in 13 experiments involving seven rats. Values shown are means (±SE) for the within-rat comparisons. * , $P < 0.01$; **, $P < 0.001$; paired $t$ tests.

The four training trials as evidenced by a decrease in the average latency for trials 3 and 4 combined versus that for trials 1 and 2 combined. The groups did not differ significantly on the first trial or on average latencies across trials. The scores for the probe trial were statistically different ($P = 0.015$; Mann-Whitney $U$ test), with the drug-treated rats requiring less time to reach the platform location. The differences between the means of the groups did not reach statistical significance ($P = 0.07$; $t = 1.51$). Given that the frequency distributions for each group were skewed, the nonparametric statistic is the more appropriate test for differences.

The behavioral effects of the centrally active modulator of glutamate receptors were also examined in an eight-arm radial maze in which rats ($n = 15$) were tested repeatedly over a period of 3 months. Daily sessions involved two episodes, in the first of which four arms were blocked. The rats were allowed to enter the open arms to retrieve a food reward (chocolate chips) and then returned to their home cages. Six or 8 hr later they were returned to the maze, which now had all arms open but contained rewards only in the previously blocked arms (blocked arms were randomized across days). The number of incorrect entries (i.e., armed entered more than once) and the number of correct choices made before a reentry occurred were recorded. Previous studies indicate that rats show excellent retention on tests of this kind at 4–6 hr with an evident decay in memory thereafter (11, 12). This occurred in the present experiments as evidenced by an increase in reentry errors from $1.1 ± 0.6$ to $1.6 ± 0.4$ (mean ± SD) in tests carried out with 6- vs. 8-hr delays between sessions ($P < 0.01$; paired two-tailed $t$ test; six experiments at each delay). The experimental question investigated with the radial maze was whether the glutamate receptor modulator would prolong the duration of memory. Fifteen rats were given sham or saline injections (4 days), vehicle injections (6 days), or drug injections (6 days) on alternate days 30 min before the first trial and were then tested 8 hr later. Fig. 2C summarizes the results; the rats exhibited substantially better retention on drug-injection days than they did on control injection days ($P < 0.001$; paired $t$ test). This was equally true for both reentry errors ($t(14) = 7.54$) and for number of correct choices ($t(14) = 5.53$) before an error. The effect held for 14 of the 15 rats—the lone exception exhibited excellent retention on control days and thus had little room for improvement. Since the rats had extensive experience with the maze before the maze was reentered with drug, it can be assumed that they were thoroughly familiar with the procedural aspects of the task. Moreover, there was no improvement over the several weeks of testing with control and drug injections, indicating that the experimental compound was not facilitating performance by promoting the encoding of “rules” pertinent to the task.

**DISCUSSION**

These results indicate that facilitation of glutamatergic transmission causes a general improvement in memory encoding. The first two studies used a suboptimal number of training trials to test whether the experimental treatment would reduce the amount of training needed for stable memory. The two paradigms involved different rewards (food, escape), sensory cues (odors, spatial relationships), and locomotor behaviors (approach and nose poking, swimming). The third paradigm tested for effects on the duration of memory and involved still another set of behaviors. In all instances, the rats injected with drug prior to initial learning exhibited substantially better performance on a subsequent memory test. It is noteworthy that learning in the three behavioral tasks is dependent on the hippocampus (13–15) and is likely to involve cortical circuitry as well. AMPA receptors, the target of the experimental manipulation, are concentrated in these regions (16).
That facilitation of excitatory receptors enhances memory has an interesting counterpart in the effects caused by manipulations that facilitate inhibitory receptors. Benzodiazepines, which augment GABA_A receptor-mediated hyperpolarizing currents, are known to produce anterograde amnesia in animals and humans (17, 18). Possibly then, the balance of fast excitatory transmission vs. the slower inhibitory responses affects the amount of training needed to form memory. This relationship could reflect an influence of excitation/inhibition on the rate at which experienced ani-
mals process information in the constrained environment of a learning task; i.e., in circumstances in which pertinent cues and pertinent responses are unambiguous and familiar. It is also possible that the balance of excitation vs. inhibition affects learning by an action on the machinery that encodes memory. Enhanced GABAergic transmission impedes the induction of LTP (19), while facilitation of excitatory receptors promotes the potentiation effect (1). This latter result presumably reflects the effects of the greater depolarization elicited by afferent bursts in the presence of the drug on the voltage-sensitive N-methyl-D-aspartate (NMDA) receptors; i.e., by enhancing the response of target cells to brief stimulation episodes, drugs that modulate the AMPA receptor increase the magnitude of the NMDA receptor-mediated currents that trigger LTP. Thus, if LTP is a substrate of memory, then enhancement of AMPA receptors would be predicted to improve learning, the result obtained in the present study. Since the stability as well as magnitude of LTP varies depending on the amount of afferent stimulation used for induction (20) and may differ across brain subsystems (9, 21), it is conceivable that the potentiation effect participates in both the decremental and stable forms of memory studied in the present experiments.

This research was supported by grants from the Air Force Office of Scientific Research (F49620).