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Modulation of Long-Term Potentiation by Peripherally Administered Amphetamine and Epinephrine

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Long-term potentiation (LTP) has received considerable attention as a neurophysiological model for studying the biology of memory. The present experiments examined the susceptibility of LTP in the dentate gyrus to modification by peripheral injections of amphetamine and epinephrine. Both drugs enhanced the development of LTP in a dose-related manner comparable to that seen previously in behavioral studies. Such results suggest that the development of this long-lasting electrophysiological change can be regulated by peripheral catecholamine levels in a manner analogous to that seen in behavioral studies of memory.

INTRODUCTION

There are now many examples of anatomical, biochemical, and physiological nervous system alterations produced by training, differential experience, or acute manipulations (e.g. refs. 2, 14, 21, 28, 30; cf. ref. 29). Long-term potentiation (LTP) is one such phenomenon; it is characterized by a long-lasting alteration in a monosynaptic evoked response after one or a series of high-frequency stimulation trains[4,5,9,10]. This neurophysiological model of memory has primarily been examined in hippocampal commissural and Schaeffer collateral systems, and in the entorhinal cortex–dentate gyrus perforant path. LTP has been proposed as a model for a process underlying memory storage because of its rapid onset (0–3 min) and long duration (months)[14,25].

Considerable evidence supports the view that both central and peripheral adrenergic systems can enhance memory storage processing[15,16]. In particular, such results suggest that peripheral adrenergic systems may facilitate the development of the central nervous system modifications responsible for storing new information. A demonstration of a relationship between those systems which modulate memory and those systems which exhibit long-lasting neuronal change might facilitate attempts to understand the neurobiology of memory. Recently, we obtained preliminary evidence that amphetamine can affect LTP under some conditions[8]. In the present experiment, we demonstrate that peripheral injections of either amphetamine or epinephrine can enhance the extent of LTP observed after high-frequency stimulation trains. The dose–response characteristics are remarkably similar to those observed in many behavioral memory facilitation studies[17,18,20,22,31].

MATERIALS AND METHODS

Male Sprague–Dawley rats (Dominion Laboratories, 300–450 g) were individually housed and were maintained on a 12 h light–dark cycle (on: 08.00–20.00 h). Under Nembutal anesthesia (45 mg/kg), stimulation and recording electrodes were placed stereotaxically just dorsal to the perforant path and dentate gyrus granule cell layer, respectively. The electrodes were then each lowered to optimize the population spike amplitude obtained with

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test pulses. The evoked responses were amplified (Grass Wide Band AC EEG preamplifier Model 7P5B and DC amplifier 7DAF; band pass 1–3000 Hz) and stored on an oscilloscope (Tektronix Model 5111). Each population spike measurement was visually averaged, based on an oscilloscope tracing of 10 consecutive superimposed traces. We have since replicated the main findings reported here using a microcomputer-based averaging system[7]; computer-plotted evoked responses are shown for illustrative purposes in Fig. 1.

On the basis of a series of pilot studies, the experimental procedures were selected to accommodate the pharmacological manipulations. First, to reduce the variance in such pharmacological studies, we found it necessary to select an arbitrary standard evoked response for baseline measurements. Therefore, the test pulse intensity was chosen for each preparation such that the population spike amplitude was equal to the EPSP amplitude (see Figure 1, top). Second, we found that the population spike amplitude diminished over the first several minutes of test pulse administration (at 1 every 30 s) and then stabilized; such findings have been reported by others[12,32,34]. To anticipate this decrement within our procedures, we began each preparation with a test pulse intensity set so that the population spike amplitude was 150% of

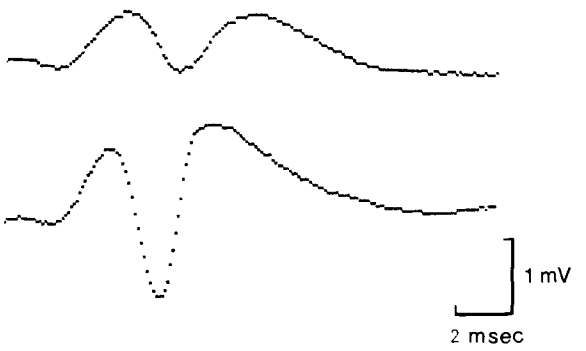

Fig. 1. Evoked responses recorded in the dentate gyrus after test pulses administered to the angular bundle. The top trace illustrates an example of a baseline response established prior to high-frequency stimulation. Note that the test pulse amplitude was adjusted in each preparation such that the population spike amplitude (downward deflection) was equal to the initial rise (EPSP). After this baseline evoked response was obtained, the evoked response did not change significantly with time or drug injection (cf. small bars in Fig. 2). The bottom trace is an example of a potentiated response (same test pulse parameters as above) recorded 20 min after the high-frequency train. This example exhibited a 128% increase in population spike amplitude.

the EPSP amplitude. The test pulses were then administered at the rate of 1 every 30 s throughout the remainder of the experiments, whether or not data were being collected, to add to the stability of the evoked response[24]. On the basis of the 10 evoked responses collected between minutes 15 and 20, small adjustments in test voltage were made at this time, if necessary, to accomplish this baseline evoked response. Each animal then received an injection (i.p.) of saline (n = 22), D-amphetamine sulfate (Sigma; 0.001, 0.01, 0.1, 1.0, 3.0, or 10.0 mg/kg; n's = 5, 5, 6, 8, 6 and 5, respectively) or epinephrine bitartrate (0.01, 0.1, 1.0, 2.0 mg/kg; n's = 4, 15, 4 and 4, respectively). Each day's preparations (4–6 animals) included saline control animals. Also, because only one epinephrine dose (0.1 mg/kg) appeared to affect potentiation, additional animals which received this dose were included on each day when other epinephrine doses were tested. Test pulses were continued for an additional 20 min after injection. The evoked responses to the 10 test pulses between minutes 15–20 after injection were used as a measure of possible drug effects on the evoked response. If necessary, small changes in the population spike amplitude were once again reversed at this time by adjusting the test voltage. This final intensity was used throughout the remainder of the procedures. To document stability of this evoked response, and to evaluate further possible drug effects at delayed times, 3 additional groups (n's = 6) received injections of saline, amphetamine (1.0 mg/kg) or epinephrine (0.1 mg/kg) but were not given high-frequency stimulation; these groups received only test pulses (1 every 30 s) throughout the time period studied in the experimental groups. As described below, the results obtained with these control groups, as well as the prepotentiation values, did not change over time or in response to the drug injections. Thus, these procedures resulted in a preparation with which it was possible to evaluate drug effects on LTP against a baseline evoked response which was stable and did not itself respond to the pharmacological manipulations.

To produce potentiation, each experimental animal received 10 trains of high frequency electrical stimulation (test voltage intensity [4–20 V], 200 Hz, 100 s; one 0.4 s train every 10 s)[9,24]. Our pilot studies indicated that these stimulation procedures, combined with the voltage used to establish the baseline

evoked response, resulted in 50–100% increases in the population spike amplitude. Additional increases can be obtained, for example, by increasing the voltage or by administering additional high-frequency trains. Thus, the specific conditions employed here result in reliable but submaximal LTP, attributes which may be important in studies of pharmacological enhancement of LTP.

Test pulses were resumed at the rate of 1 every 30 s, beginning 30 s after the end of the high frequency trains. Potentiation was assessed by measuring the percent change in the population spike amplitude as observed in the 10 evoked responses collected between minutes 15 and 20 after the high-frequency stimulation trains. The 15–20 min interval was chosen because the procedures could be completed in all animals without the need for supplemental anesthesia injections, which we judged to be a potential confound. Although not systematically studied, the evoked response was examined in several preparations at times up to 2 h later; these later evoked responses did not appear to differ from those collected at the 15–20 min interval.

RESULTS

In animals which received the high-frequency stimulation trains, the population spike amplitude was initially increased and then grew still larger over the first 1–3 min after the high-frequency train. An example of the potentiated waveform is shown in Fig. 1 (bottom). The results shown in Fig. 2 are based on measurements of the 10 pulses (1/30 s) taken between 15 and 20 min after the high-frequency train. Both adrenergic drugs produced an inverted-U dose-response curve for enhancement of LTP. Amphetamine resulted in significant enhancement in the extent of potentiation of the population spike amplitude (vs saline controls, *t*-tests) at 4 of the 6 doses; significant differences were observed in animals which received amphetamine at doses of 0.01 ($P < 0.01$), 0.1 ($P < 0.05$), 1.0 ($P < 0.01$) and 3.0 ($P < 0.001$) mg/kg. Higher (10.0 mg/kg) and lower (0.001 mg/kg) doses were ineffective. Similarly, epinephrine injections significantly enhanced the growth in population spike amplitude at a dose of 0.1 mg/kg ($P < 0.05$) but not at higher or lower doses.

These effects on the amplitude of the population

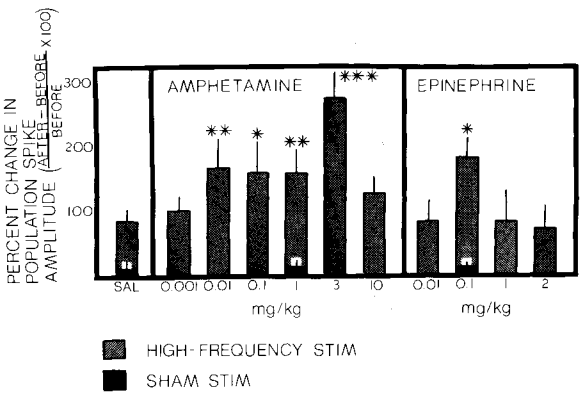

Fig. 2. Percent change (means ± S.E.M.) in population spike amplitude 20 min after high-frequency stimulation. A peripheral injection of either amphetamine or epinephrine enhanced the degree of long-term potentiation in an inverted-U dose–response manner. The small internal black bars (saline, 1.0 mg/kg amphetamine, 0.1 mg/kg epinephrine) illustrate the stability of the evoked response in drug-tested animals which did not receive a high-frequency stimulation train (vs saline group: * $P < 0.05$; ** $P < 0.01$; * $P < 0.001$; two-tailed *t*-tests).

spike after high-frequency stimulation appear to reflect interactions with LTP itself and not an action on the evoked response. There were no significant drug effects on the evoked response prior to high-frequency stimulation (matched *t*-tests, $P > 0.2$ in each case). Also, in other groups of animals (n's = 6), we examined possible changes in the evoked responses of injected animals which did not receive the high-frequency train. The population spikes measured 15–20 min following sham-stimulation (30–40 min after injection) were not significantly altered from baseline values (saline: 11 ± 10%; 1.0 mg/kg amphetamine: 13 ± 9%; 0.1 mg/kg epinephrine: 15 ± 6%; see sham groups, Fig. 2). Thus, the drug effects shown in Fig. 2 appear to reflect modulation of the neurobiological events underlying long-term potentiation.

DISCUSSION

The present results suggest that peripheral catecholamines may be important in modulating LTP, as they are for modulating memory. It is important to note that the drug injections did not themselves alter the evoked response. Also, in our preliminary studies, we found that larger degrees of LTP can also be produced, in the absence of pharmacological treatment, by increasing the intensity or number of high-frequency stimulation trains. Thus, one interpreta-

tion of our findings is that peripheral injections of epinephrine or amphetamine might act by reducing the prerequisites for establishing a particular level of LTP. According to this view, the findings suggest that peripheral catecholamines may regulate the extent to which synaptic transmission in the central nervous system results in durable modifications of functional connectivity.

Epinephrine apparently does not cross the blood–brain barrier in large amounts[1] and yet the peripherally injected amine can enhance both LTP and memory. Amphetamine enhancement of LTP may also be mediated by peripheral adrenergic actions. Recent findings suggest that memory facilitation with amphetamine may be mediated by peripheral and not central actions of the drug[26,27]. Also, amphetamine apparently does not enhance LTP if applied to in vitro rat hippocampus preparations[13]. Although the mechanisms by which peripheral catecholamines can enhance LTP and memory are unknown, it is important to note that peripheral epinephrine does affect several brain functions. For example, epinephrine injections can result in alterations of brain electrographic arousal[3], brain noradrenergic activity[18], and the development of kindled seizures[33]. Thus, the present findings add to the list of central nervous system effects of peripheral catecholamines and add to our need to understand the undefined processes which mediate the central actions of peripheral amines.

There are now several anatomical and biochemical correlates of LTP[2,6,11,19,23,25]. The present results may be useful in promoting a continued analysis of these correlations by providing conditions under which the parameters of the potentiating stimulation train are held constant but the extent of potentiation can be varied pharmacologically. Thus, it may be possible to distinguish between anatomical and biochemical responses to high-frequency stimulation trains which do and do not mediate long-term potentiation. Such procedures may permit more rigorous tests of the importance of the correlates to the mechanisms underlying LTP.

The analogies between the results described here and those obtained with these drugs in behaviorally assessed memory experiments are quite striking: (1) amphetamine and epinephrine can enhance both LTP and memory, (2) the dose–response curves have an inverted-U form, as they do in behavioral experiments, and (3) the optimal doses for enhancement are similar to those obtained in behavioral experiments. Thus, the brain changes underlying both memory and LTP may be regulated, in part, by the activity of peripheral adrenergic systems.

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