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Modulation of Long-Term Potentiation: Effects of Adrenergic and Neuroleptic Drugs

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DUNWIDDIE, T. V., N. L. ROBERSON AND T. WORTH. *Modulation of long-term potentiation: Effects of adrenergic and neuroleptic drugs*. PHARMAC. BIOCHEM. BEHAV. 17(6) 1257–1264, 1982.—A variety of drugs which either mimic or antagonize the effects of norepinephrine and dopamine were tested for their ability to modulate long-term potentiation (LTP) in the rat hippocampus in vitro. Neither administration of norepinephrine, amphetamine or adrenergic antagonists, nor pretreatment with reserpine or DSP4 (which selectively destroys noradrenergic afferents to the hippocampus) had any significant effect on the magnitude of LTP. Isoproterenol, a β-adrenergic receptor agonist, was able to partially block LTP, but this did not appear to be due to a direct action of isoproterenol on LTP. Neuroleptic drugs such as trifluoperazine were able to block LTP almost completely; however, this action was not stereospecific. Other dopamine antagonists such as sulpiride had no effect on LTP. The ability of neuroleptics to antagonize LTP was more closely related to their ability to block calmodulin than to their relative potencies as dopamine antagonists. It would appear that neither norepinephrine nor adrenergic antagonists influence the amount of LTP elicited by repetitive stimulation; however, drugs which have been shown to interfere with calmodulin-mediated cellular processes do antagonize this phenomenon.

Long-term potentiation (LTP) Learning and memory Catecholamines Neuroleptic drugs α-Blockers β-Blockers α-Agonists β-Agonists Hippocampus in vitro

BEHAVIORAL studies of the neuropharmacology of learning and memory have repeatedly implicated catecholamine neurotransmitters as modulators of memorial processes. It has been suggested that the noradrenergic input from the brainstem nucleus locus coeruleus to cortical and other forebrain regions plays an important role in the storage of information. However, while pharmacologic manipulation of catecholamine systems can either facilitate or disrupt the subsequent performance of a learned task [1, 20, 28, 30, 35], a consistent role for catecholamines has yet to be defined. It is unclear whether catecholamines affect memory per se, modulate reinforcing aspects of learning [8], alter emotional elements of a learning situation [29], or facilitate the selective filtering of sensory information essential to learning [28]. Some evidence suggests peripheral as well as central adrenergic activity can affect memory processes as well [20].

One possible way in which adrenergic drugs might affect memory is via a direct action upon the neurophysiological substrate for memory. Although many candidates for such a process exist, the long-term potentiation (LTP) of synaptic responses in brain regions such as the hippocampus appears best suited for such a role (see [11,27] for reviews). LTP is an enduring increase in synaptic efficacy that occurs following repetitive stimulation (as few as 10–20 impulses), which can persist for weeks or even months in intact animals [10]. It can be elicited in several synaptic pathways in the hippocampus [4, 5, 13], a structure frequently associated with memory functions, but can also occur following high-frequency stimulation in other systems as well [6]. The characteristics of LTP appear quite similar in the intact unanesthetized animal [4,7] and in isolated in vitro preparations such as are used in the present experiments [13, 36, 37]. Because of these properties, it has been frequently been suggested that a similar change in synaptic efficacy, occurring under more physiological conditions, underlies many of the long-term changes in behavior which accompany learning.

Because of the possible role of LTP as a substrate of learning and memory, we have investigated whether adrenergic drugs can modulate long-term potentiation in the in vitro hippocampus. We have chosen this preparation as a test system for several reasons. First, LTP can be reproducibly and reliably elicited from hippocampal slices, facilitating quantitative comparisons of LTP under various conditions. Second, we have studied in considerable detail the adrenergic pharmacology of the hippocampus in vitro [31, 32, 33]; the effects of perfusion with selective α- and β-receptor agonists and antagonists have been well characterized. Third, an in vitro preparation permits quantitative estimates to be made of drug concentration; hence, it is often possible to relate effects to particular receptors or receptor subtypes. Because the slice is isolated from the rest of the

brain (and animal), it is possible to rule out indirect effects mediated via other brain regions, changes in vascular perfusion, anesthetic level, etc.

In addition to investigating the effects of adrenergic drugs on LTP, a second aspect of the present experiments was to investigate in greater detail the possible role of calcium and calmodulin in LTP. Several investigators have demonstrated that calcium or other cations which can substitute for it are essential in order to elicit LTP [14, 15, 41]. In addition, brief perfusion of slices with medium containing elevated Ca^{++} can apparently elicit an LTP-like process, even in the absence of high frequency stimulation [38]. Treatment of hippocampal membranes with Ca^{++} can increase the number of glutamate binding sites [2], as can electrical stimulation of the intact slice [3]. Based upon these observations, Lynch and co-workers have suggested that calmodulin in some way links a Ca^{++} influx associated with high frequency stimulation, and the subsequent increase in the efficacy of synaptic transmission. The antagonism of LTP by trifluoperazine [18] provides further indirect support for this hypothesis. Trifluoperazine (TFP) binds to calmodulin and blocks its activity in vitro [25,26]. Therefore, it has been suggested that TFP blocks LTP by interfering with a calmodulin-dependent phosphorylation step which may underlie LTP. However, TFP is a potent neuroleptic drug which can block dopaminergic, and perhaps serotonergic and adrenergic receptors, in the concentrations used by Finn *et al.* [18]. Therfore, we examined the effects of several drugs which vary in their relative potencies as neuroleptics and calmodulin antagonists in an attempt to dissociate the two major actions of these drugs.

METHOD

These experiments were conducted on hippocampal slices from male Sprague-Dawley rats weighing between 150–250 g and obtained from Charles River. Coronal slices of the mid-septo-temporal region of both hippocampi were prepared as described previously [12,13].

In the present experiments, recordings were made via 3–4 MΩ glass microelectrodes filled with 3 M NaCl and placed under visual guidance. Bipolar stimulation pulses were delivered in the stratum radiatum near the border of CA1-CA2 to elicit orthodromic synaptic responses in the CA1 region. Simultaneous recordings were made from each slice in stratum radiatum (to measure the extracellular field EPSP) and in stratum pyramidale (population spike response). All responses were stored on computer disk, and analyzed on- and off-line for changes in the evoked responses.

In all of the pharmacological experiments, slices were either incubated in a bath containing the test drug, or were superfused first with control medium, then with medium containing the test drug as described previously [31]. Under either condition, slices were tested 1/min with test pulses sufficient to elicit a population spike whose amplitude was 20% of the maximal response which could be elicited at those electrode positions. If a drug caused a change in the response amplitude, the stimulation voltage was adjusted to elicit a population spike which was 20% of the new maximal response. This adjustment was typically required with the adrenergic agonists, which frequently elicit changes in spike amplitude (see [31,32]). When 5 stable control responses had been recorded, a 500 Hz, 0.5 sec train was interposed, and the slice was tested 1/min for at least the next 10 min. The average post-train response was calculated from a stable portion of the post-stimulation test period, usually at 3 min and sometimes as long as 10 min following the stimulation. This period varied because of differing amounts of short-term facilitation elicited by the potentiation train. Following this initial decline, the residual increase in amplitude over control responses was virtually non-decremental, and had all the characteristics associated with LTP [11].

Control (non-drug) experiments were conducted on slices randomly selected from every animal; usually between 1/4–1/3 of the slices from every preparation were used as controls. Because there was no significant between-animal variability in the controls (ANOVA; $p>0.5$), they were pooled. However, perfused and non-perfused controls were not grouped because the former showed somewhat less (although not statistically significant) potentiation.

In some experiments, catecholamines were depleted by pretreating rats with reserpine (5 mg/kg, IP) 12 hours before the beginning of the experiment. In another series of experiments, animals were injected IP with freshly prepared DSP-4 (N-(2)chloroethyl)-N-ethyl-5-bromobenzylamine; 50 mg/kg), which produces large and essentially irreversible decreases in norepinephrine (NE) but not dopamine (DA) in mice and rats [24]. These animals were tested 30–40 days following the lesion, at which time hippocampal concentrations of NE are still highly depressed [16].

Drugs were obtained from the following sources: norepinephrine; isoproterenol; d-amphetamine (Sigma); clonidine (Boehringer Ingelheim); l-α-methyl-norepinephrine (Sterling-Winthrop); phentolamine; reserpine (CIBA-GEIGY); trifluoperazine (Smith, Kline and French); haloperidol (McNeil Pharmaceutical); α- and β-flupentixol (Lundbeck and Company); S-sulpiride (Ravizza); timolol (Merck); and DSP4 (Dr. G. Jonsson).

RESULTS

Potentiation Under Control Conditions

In order to quantitatively compare the amount of LTP observed under various conditions, we conducted preliminary studies to determine the most reliable parameters for eliciting LTP. Complete input-output (I-O) curves (stimulation voltage vs evoked response amplitude) were made by testing the response at 20 sec intervals with pulses of varying intensity. Following this, the stimulation voltage was reduced so as to elicit a population spike with an amplitude 20% of the asymptotic maximal response, and the response was then tested 1/min for 5 min. A stimulation train (500 Hz, 0.5 sec duration) was then delivered at the same intensity, and the response tested 1/min for 20 min. Another complete I-O curve was then acquired and the degree of potentiation of the field EPSP and population spike was determined at the various test voltages. A total of 10 slices were tested in these preliminary experiments.

Averaged input-output curves for the EPSP and the population spike are shown in Fig. 1. The percent potentiation at each stimulation intensity is also illustrated (Fig. 1C). LTP was characterized by an increase in response amplitude which was apparent throughout the range of test voltages, although the percent increase varied considerably. Since the coefficient of variation for both EPSP potentiation, and potentiation of the population spike were lowest near the bottom of the voltage scale, we routinely set the stimulation voltage so as to elicit a 20% maximal population spike response prior to potentiation in all the succeeding experiments.

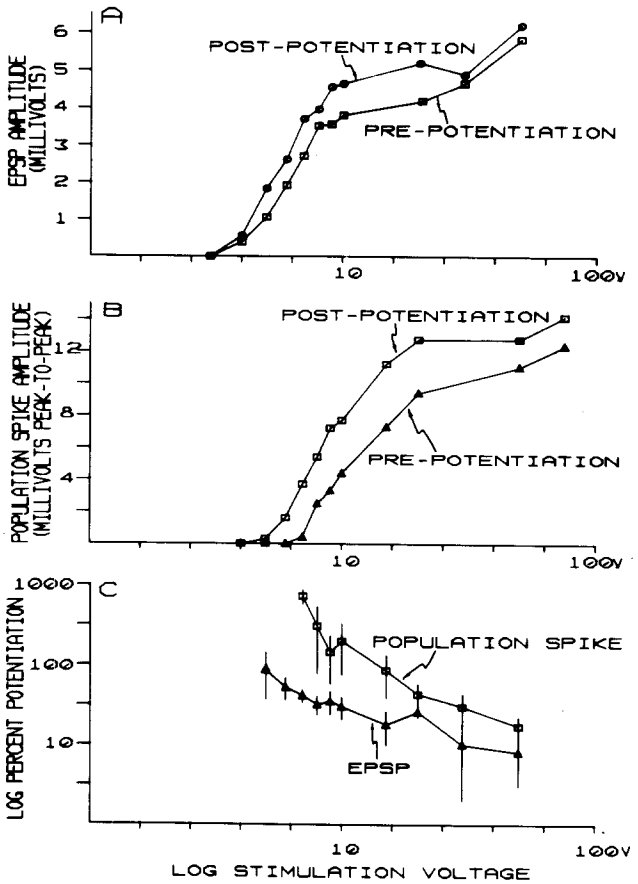

FIG. 1. Characteristics of LTP in control slices. Input-output curves (voltage vs response amplitude) are shown for the field EPSP (A) and the population spike (B) before and after a 500 Hz, 0.5 sec stimulation train. Groups of slices were tested 5 min before the train (pre-potentiation), and 20 min following the train; each point represents the mean of 4–5 slices tested at each intensity. In (C) the increase in response at each voltage was expressed as a percent of the response prior to the train for both the EPSP and population spike. The degree of potentiation varied from 10% to nearly 1000%, depending upon the pre-stimulation response amplitude; the absolute increase was roughly constant throughout the entire range of response amplitudes (cf. (A) and (B). Note the log scale for stimulation voltage in all sections, and for the percent potentiation in (C) as well.

As can be seen in Fig. 2, a high frequency stimulation train elicits changes in several aspects of the evoked response which persist for some time following the train. The population spike (A1), the peak amplitude of the field EPSP (B1, upper), and the slope of the falling phase of the field EPSP (B1, lower) all show significant increases as a result of repetitive stimulation. Although the absolute magnitude of the changes in each measure differed, we always found that changes in any one parameter were paralleled by changes in the others. Because no differences were observed between response measures, we will present only the data for the population spike in this communication.

Finally, although the 500 Hz/0.5 sec train used in these experiments elicited a fairly high and quite significant degree of potentiation, this did not appear to be a maximal response. As seen in Fig. 3, a subsequent stimulation train at the same amplitude (also 500 Hz, 0.5 sec) could elicit further increases in response amplitude.

Effects of Adrenergic Agonists and Antagonists on LTP

Previous work from our laboratory has demonstrated that adrenergic agonists produce physiological responses in rat hippocampus that are mediated via both α and β receptors [31]. Therefore, we examined the actions of 4 different classes of adrenergic agonists; selective α-receptor agonists (clonidine and l-α-methyl norepinephrine), a selective β-agonist (isoproterenol), a mixed agonist (NE) and an indirectly-acting agonist (amphetamine). In these experiments, the medium in the recording chamber was simply replaced with medium containing the drug, but was not perfused. The concentrations selected were sufficient to elicit maximal drug responses, based upon dose-response curves obtained previously. Since all of these drugs produced changes in population spike ampltiude, stimulation voltages were routinely adjusted following the drug response so that the population spike amplitude remained at 20% of maximal. The results of these experiments are illustrated in Fig. 4.

Norepinephrine (25 μM) and amphetamine 20 (μM) were both tested for their effects on LTP. This concentration of NE appears to activate α- as well as β-adrenergic receptors in hippocampal slices [31] and most commonly produces increases followed by decreases in population spike ampltiude. Amphetamine appears to activate these same receptors by releasing NE from endogenous stores [39], because amphetamine responses can be antagonized by adrenergic blockers [32] or by lesions of adrenergic afferents to the hippocampus [16]. However, neither NE nor amphetamine produced any significant effect upon LTP.

Clonidine, an α_2-receptor agonist, reduced the amount of post-train potentiation by about 40%, but this decrease was not statistically significant. l-α-Methyl-norepinephrine, another potent α_2-agonist, produced a non-significant increase in LTP. Isoproterenol, a β-selective receptor agonist, caused a significant reduction in the amount of LTP which was observed. However, isoproterenol altered several aspects of the physiological response to high-frequency stimulation. Whereas all 30 control slices showed facilitated responses 1 min following the train, 3/17 of the isoproterenol-treated slices demonstrated no detectable synaptic response 1 min following the stimulation train; another 3/17 had extremely small responses. In these 6 slices, test response amplitudes recovered towards baseline, but none showed LTP. These isoproterenol-treated slices were only the ones which ever showed depressed responses 1 min following the stimulation train. In isoproterenol-treated slices which did not show this post-train depression, the pattern and degree of LTP was virtually identical to that seen in controls (Fig. 5).

These data suggest that adrenergic agonists do not appear to interact directly with LTP. However, we also tested whether endogenous NE might play a role in eliciting LTP. Therefore, the effects of adrenergic antagonists were investigated as well (Fig. 6). Timolol, a selective antagonist of hippocampal β-receptors [31] had no effect on LTP. Phentolamine, a selective α-receptor antagonist, produced a fairly large, although not significant decrease in the amount of LTP. However, this effect was not reversed by α-receptor agonists; in fact, the effects of clonidine and phentolamine were additive, and resulted in an almost complete blockade of LTP. In other experiments, NE levels in the hippocampus

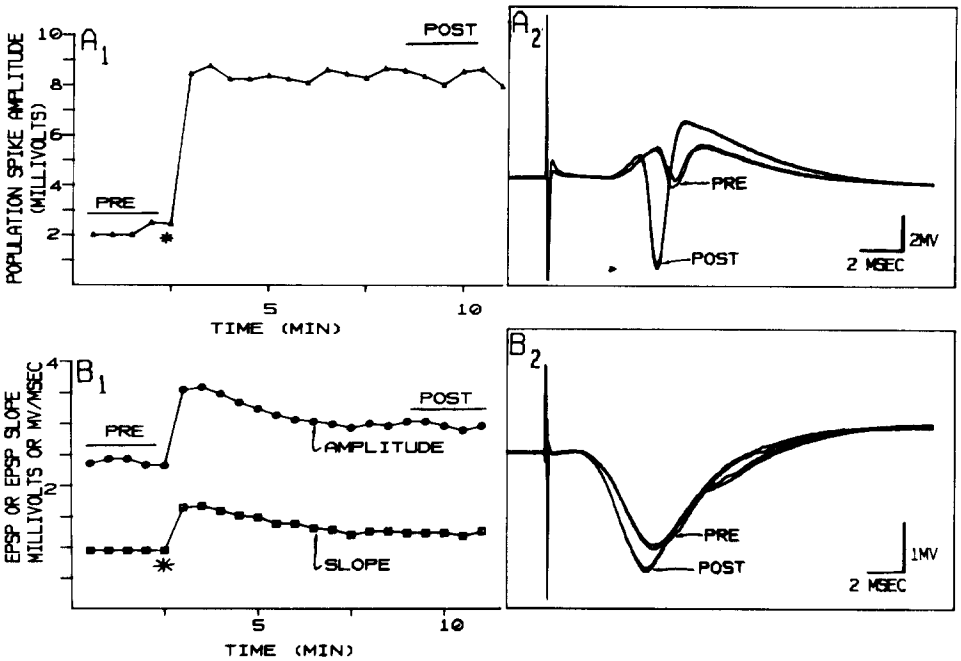

FIG. 2. Time course of responses to high-frequency stimulation. (A). The time course of increases in the population spike amplitude are illustrated in (A_1). The 500 Hz, 0.5 sec stimulation train was delivered immediately following the response marked with the asterisk. This response was somewhat atypical in that there was little if any post-train decline in the potentiated response. (A_2) illustrates the fact that significant changes occurred in several aspects of the evoked field response recorded from stratum pyramidale. The 95% confidence limits for averages of 5 responses (indicated by the bars over points in (A_1) are shown; throughout most of the response the 95% limits are so close as to appear as a single line. There is a highly significant increase in the rising phase of the evoked response, the amplitude and latency to the sharp, negative-going population spike, and the amplitude of the following positivity. (B) Records from different slice than in A illustrate the time course of changes in the amplitude and slope of the field EPSP recorded extracellularly from stratum radiatum. The slope was potentiated by 28% during the 5 "post" responses (Student's $t=20.0; p<0.001$), whereas the maximum amplitude was potentiated by 24% (Student's $t=16.8$; $p<0.001$). The initial decline in each of these parameters was more typical of the responses than was the nondecremental response in (A). The initial component declines fairly rapidly, leaving a stable level of LTP (see [11] for a quantitative discussion of this initial decline). The averaged responses in (B_2) demonstrate the types of changes which occur in the evoked field EPSP in response to a potentiation train.

were reduced by pharmacological treatment prior to slice preparation. DSP4 lesions, which reduced hippocampal NE levels by an average of 72% (see [16]), had no effect on LTP. Depletions due to reserpine pretreatment also had no influence on potentiation.

Effects of Neuroleptic Drugs on LTP

The neuroleptic drug trifluoperazine (TFP) has been shown to antagonize LTP [18]. In our experiments, we sought to test whether this was a general property of drugs which affect calmodulin, or of drugs which block dopamine and/or other catecholamine receptors. Because the effects of these agents on normal synaptic transmission were unknown, these experiments were conducted on superfused slices. In this way, the effects of antagonists could be characterized under more stable recording conditions. Some neuroleptic drugs (e.g., haloperidol) were found to have such pronounced effects on synaptic transmission that they could not be unequivocally tested for interactions with LTP. However, the drugs used in the experiments described below generally produced little if any change in the control responses.

The effects of neuroleptic drugs on LTP are summarized in Fig. 7. As reported previously, 40 μM TFP almost completely abolished LTP. Unlike isoproterenol, TFP treatment did not result in a post-train depression of synaptic responses. Instead, there was a simple reduction in the magnitude of stimulation-induced changes, with no apparent effect on the general pattern or time-course of the changes (Fig. 5). This effect of TFP was shared to a lesser degree by both the α- and β-isomers of flupentixol. However, while the α-isomer of flupentixol has more than a 100-fold greater potency in displacing radiolabelled spiroperidol from dopamine receptors than the β-isomer, no comparable stereoselectivity was apparent in the LTP antagonism. At most, the α-isomer is approximately 2-fold more potent than is the β-isomer, since 100 μM β-FPT was roughly equipotent with 50 μM

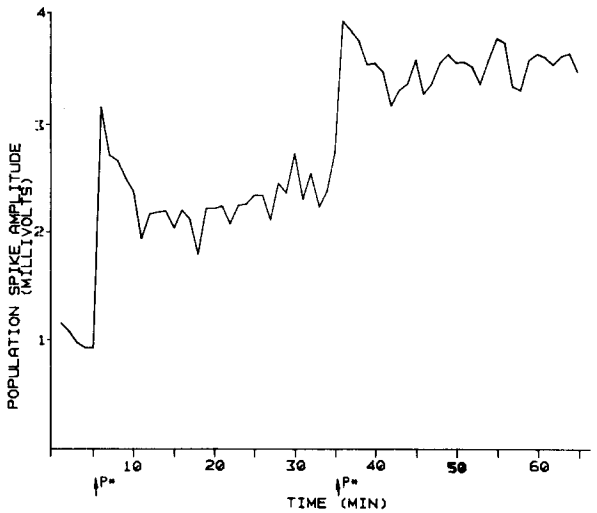

FIG. 3. Effects of repeated stimulation trains. The changes in population spike amplitude (recorded from a single slice) in response to repeated stimulation trains is illustrated. The initial decline in the response following the first train (P*) lasted approximately 10 min; the residual LTP was fairly stable over the suceeding 20 min period. The second train (at 35 min), produced a further potentiation of population spike amplitude, although this second increase was not as large as the initial response.

DRUG	CONT	CLON	AMNE	ISO	NE	AMPH
TYPE	---	ALPHA	ALPHA	BETA	MIXED	INDIR
CONCENTRATION	---	10 μM	10 μM	10 μM	25 μM	20 μM
N	30	11	7	17	13	11
T-VALUE	---	1.98	-0.65	2.28*	0.18	1.58

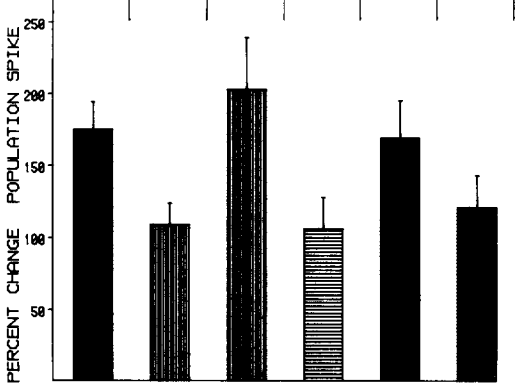

FIG. 4. Effects of adrenergic agonists on LTP. The average potentiation (±SEM) of the population spike response is shown for control slices (solid bar), and for slices pretreated with the indicated concentrations of alpha-receptor, beta-receptor, mixed, and indirectly-acting adrenergic agonists. N indicates the number of individual slices tested in each condition. The *t* values from Student's *t*-tests are shown in the bottom row. Only the ISO-induced decrease in LTP was statistically significant ($p<0.05$, two-tailed test). The pattern of drug effects on the field EPSP paralleled that seen with the population spike, although the absolute magnitude of the potentiation (e.g., average potentiation of the field EPSP was 27±6.8% for the controls), and the changes induced by the drugs were considerably smaller.

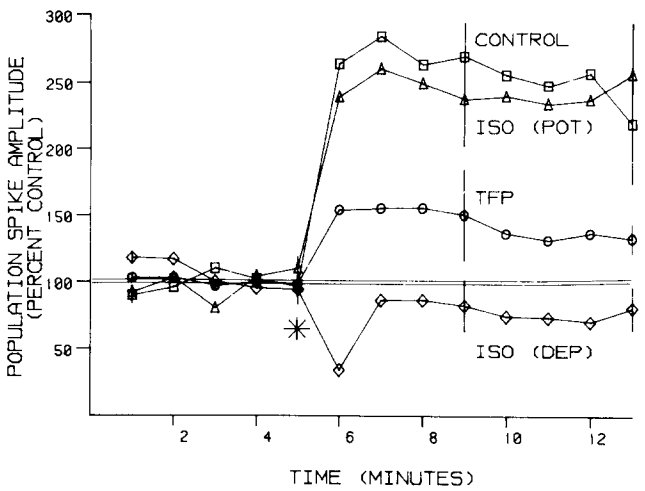

FIG. 5. Effects of isoproterenol and trifluoperazine on the time course of responses to high frequency stimulation. The mean amplitude of evoked population spike responses is shown for control slices, and for slices pretreated with ISO and TFP. Each point represents the mean response from 5–6 slices (±SEM on every 5th point). The ISO slices were divided post hoc into two groups, based upon whether the first post-train response was increased (ISO-POT), or depressed (ISO-DEP) relative to the last pre-potentiation test response. In slices that did not show immediate depression, the degree of potentiation was virtually identical to that seen in non-drug treated slices. On the other hand, TFP reduced the amount of potentiation without significantly altering the pattern of post-train changes in response amplitude.

α-FPT in antagonizing LTP. Another dopamine receptor antagonist, S-sulpiride, had no significant effect upon LTP.

DISCUSSION

Catecholamines have frequently been implicated in various aspects of learning and memory. However, it is difficult to generate a single unifying hypothesis that can adequately explain the disparate behavioral data regarding catecholamines and learning. Therefore, we have begun to investigate this question by examining the effects of a variety of treatments which will either mimic or antagonize adrenergic transmission. By doing so in an in vitro preparation, we have been able to avoid the complications introduced by the actions of these drugs on peripheral adrenergic systems. The present experiments appear to clarify several aspects of the relationship between catecholamines and LTP:

(1) NE does not appear to be required for LTP to occur. Depletion of endogenous stores (reserpine), lesions of NE-containing afferents to the hippocampus (DSP4), and blockade of adrenergic receptors (timolol, phentolamine) appear to have little or no effect on LTP. Although phentolamine produced a ca. 40% decrease in LTP, it appears unlikely that this involves an antagonism of the effects of endogenous NE at α-receptors, because this response was additive with the effects of the α-agonist clonidine. We have previously demonstrated that phentolamine can antagonize the depressant effects of clonidine on nonpotentiated synaptic re-

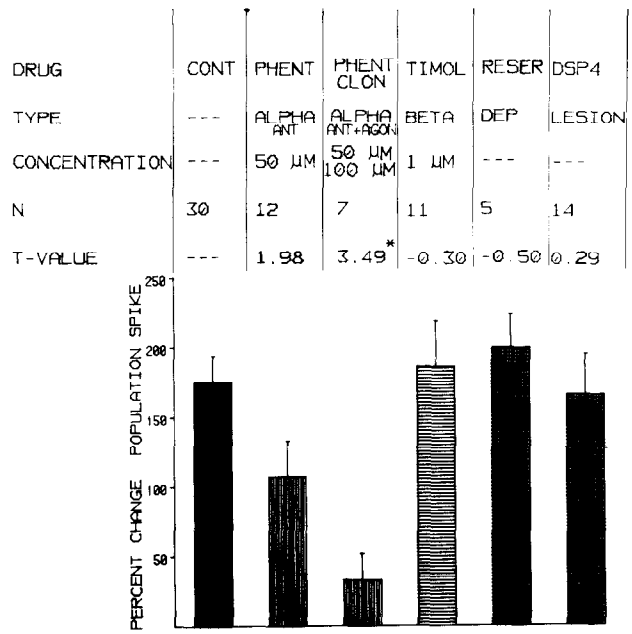

FIG. 6. Effects of adrenergic antagonists, and of prior depletion of hippocampal NE on LTP. The average potentiation of the population spike amplitude is shown for control slices (same controls as in Fig. 4; solid bar), for slices pretreated with the indicated concentrations of alpha- and beta-receptor antagonists, and for slices in which NE concentrations were reduced by reserpine or DSP4 pretreatment. In addition, the additivity of the response to an alpha receptor agonist (clonidine) and the alpha receptor antagonist (phentolamine) is illustrated. The *t*-values for each condition are shown in the bottom row. The only significant response was that elicited by the combination of phentolamine and clonidine.

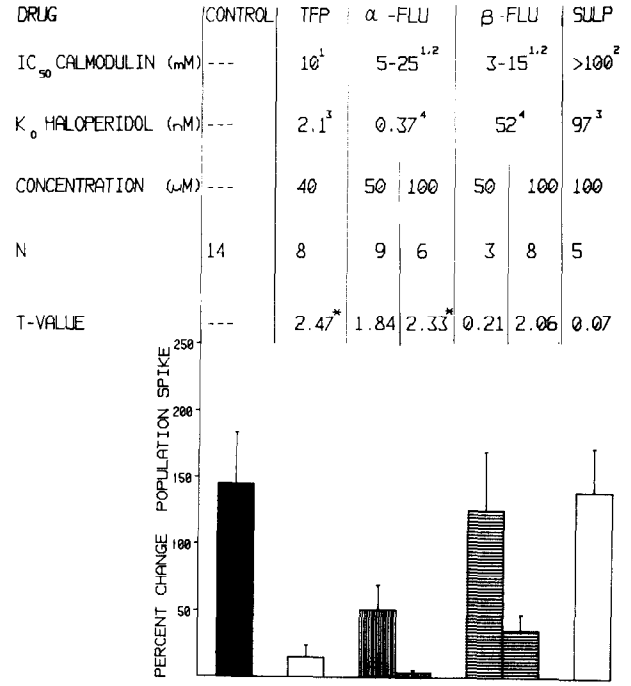

FIG. 7. Effects of neuroleptic drugs on LTP. The average potentiation of the population spike response is shown for perfused control slices (solid bar), and in slices superfused with the indicated concentrations of neuroleptic drugs. The IC_{50} values for these drugs in inhibiting calmodulin-mediated responses are taken from: [1] [40] and [2] [34]. The K_D values for the neuroleptic drugs in displacing triatium-labelled haloperidol or spiroperidol binding were taken from or calculated from data in [3] [23] and [4] [42].

sponses in hippocampus (30). If the effect of phentolamine on LTP was related to an antagonism of α-receptors, an agonist would have been expected to at least partially reverse this effect. It is perhaps more likely that this effect results from an inhibition of calmodulin by phentolamine (see next section), since phentolamine is fairly potent in this regard (IC_{50}=10 μM; [17]).

(2) NE and other adrenergic agonists do not appear to directly antagonize LTP. The only drug in this group which had any effect was isoproterenol, which produced a significant antagonism of LTP elicited by the stimulation train. The post-train depression seen in isoproterenol-treated slices was never observed either in the controls, perfused (n=14) or non-perfused (n=30), or in other drug-treated slices, but has been observed previously when a stimulation train elicits epileptiform discharges (Dunwiddie, unpublished observation). The lack of potentiation in these cases is not particularly surprising, since it has been shown that seizures in vivo can reverse the potentiation elicited by preceding stimulation trains [21]. It would appear likely that the excitatory [31] and proconvulsant [32] effects of isoproterenol in the hippocampus in vitro may lead to post-train seizures which can interfere with LTP. This type of response may not have been observed with 25 μM NE because of the opposing anticonvulsant effect of activation of α-receptors [32] at this concentration.

Thus, it would appear that catecholamines are not absolutely required for, and do not directly antagonize LTP. However, it is not clear from these experiments whether catecholamines modulate LTP in a more subtle fashion. For example, the stimuli used in these experiments were considerably above the threshold for eliciting LTP. Trains of as few as 10–20 pulses at 500 Hz can elicit some LTP under these conditions (Dunwiddie, unpublished observation). Thus, it remains to be seen whether catecholamines can affect the threshold for eliciting LTP.

These results would initially appear to contradict earlier work suggesting that destruction of noradrenergic afferents to the hippocampus can interfere with LTP [19], and that amphetamine can facilitate LTP [9]. However, both of these reports examined LTP in the perforant path input to the dentate gyrus, and tested these responses in the intact animal. Either regional differences in adrenergic modulation of LTP, or a catecholamine interaction with a non-hippocampal locus to facilitate potentiation could explain these results.

Calmodulin and the Role of Dopamine Receptors in LTP

The role of calcium (or ions which can substitute for it) in eliciting LTP has been demonstrated by several investigators [14, 15, 41], as discussed in the introduction. Because TFP can block LTP, as well as inhibit calmodulin-dependent cel-

lular processes, it has been suggested that calmodulin is directly involved in LTP. Nevertheless, neuroleptic drugs such as TFP can block a variety of cellular processes other than those mediated via calmodulin. TFP, in particular, is a potent dopamine antagonist, whose affinity for dopamine receptors (K_I=2.1 nM; [23]) is approximately 1000 times lower than the concentration needed to inhibit calmodulin (IC_{50}=10 μM; [40]) or antagonize LTP [18]. In order to determine which pharmacological action of TFP is relevant to its antagonism of LTP, we examined the effects of several other drugs which share some but not all of these actions. The results can be summarized as follows:

(1) Drugs which antagonize calmodulin block LTP. TFP, as well as both isomers of flupentixol, decrease the amount of LTP which is observed. Sulpiride, which has very little effect on calmodulin, did not alter LTP. Phentolamine, which is also able to antagonize calmodulin [17], appeared to be approximately equipotent with β-flupentixol.

(2) Affinity of neuroleptics for dopamine receptors does not predict potency in antagonizing LTP. Although dopamine antagonists such as TFP can block LTP, others such as sulpiride did not. Because sulpiride is a fairly selective antagonist for dopamine D_2 sites, it could be argued that the effect of TFP is mediated by D_1 receptor sites. However, this would appear unlikely in light of the fact that the α- and β-isomers of flupentixol, which were approximately equipotent as antagonists of LTP, differ by 2 orders of magnitude in their potency in antagonizing DA-mediated increases in adenylate cyclase activity, an effect related to the D_1 receptor [23]. Finally, the concentrations of drugs required to significantly antagonize LTP were generally 1,000–100,000 × higher than those which would be expected to block dopamine receptors.

On the basis of these experiments, it is possible to conclude that dopamine receptors are unlikely to be involved in LTP. On the other hand, they cannot provide direct evidence implicating calmodulin in LTP, since other actions of these drugs (e.g., membrane-stabilizing effects) might possibly be involved as well.

In summary, the current experiments lend indirect support to the hypothesis that LTP is mediated via a calmodulin-dependent process; drugs which interfere with calmodulin appear to block the long-term sequelae of high frequency stimulation of this preparation. To the extent to which learning involves LTP-like processes in the intact animal, these drugs would also be expected to interfere with the learning/and/or recall of a behavioral task. On the other hand, it would appear that the catecholamines NE and DA are not directly involved in LTP in vitro.

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