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2-Amino-3-phosphonopropionic acid, an inhibitor of glutamate-stimulated phosphoinositide turnover, blocks induction of homosynaptic long-term depression, but not potentiation, in rat hippocampus

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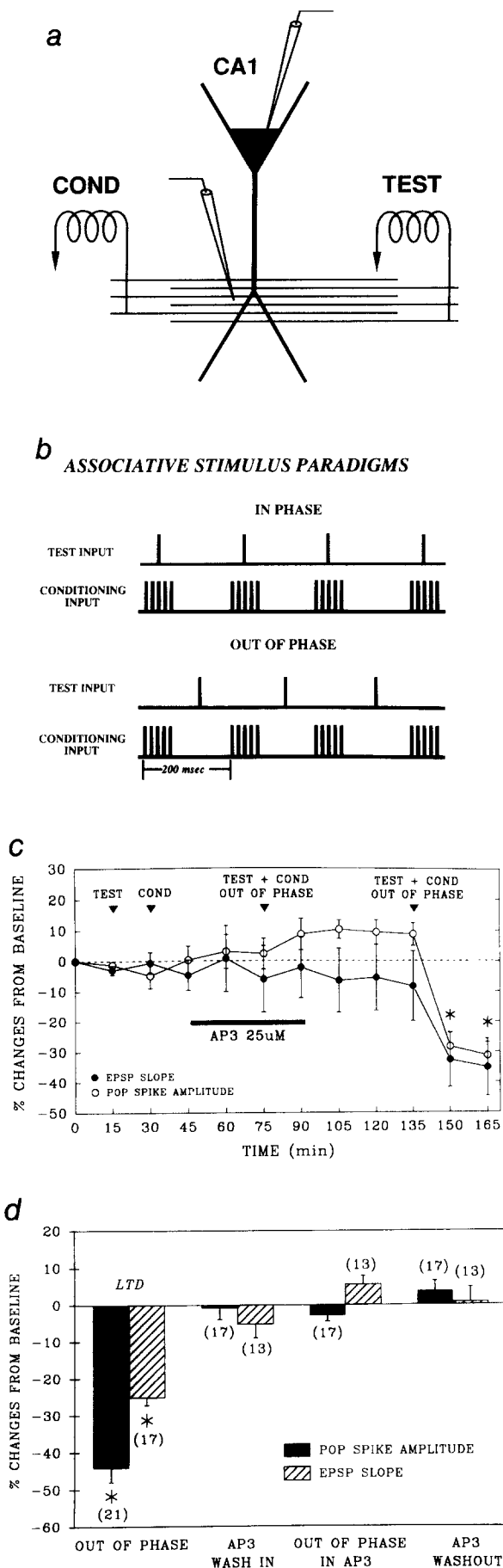
Recently, we have reported an associative long-term depression (LTD) of synaptic strength in hippocampal field CA1 that is produced when a low-frequency test input is negatively correlated in time with a high-frequency conditioning input. We have also found that pairing of synaptic activity with postsynaptic hyperpolarization is sufficient to induce LTD. We report here that 2-amino-3-phosphonopropionate (AP3), a selective inhibitor of phosphoinositide (PI) turnover mediated by the metabotropic glutamate receptor, blocks the induction of associative LTD in hippocampal field CA1, but does not impair the induction of LTP. Our data suggest that metabotropic glutamate receptor activation is involved in the induction of LTD.

The hippocampus, a cortical structure involved in learning and memory [21], is capable of exhibiting both long-term potentiation (LTP) and depression (LTD) of synaptic strength in response to certain patterns of afferent firing. LTP in the hippocampus is produced by brief, high-frequency stimulation of excitatory afferents [4]. Associative LTP is elicited when low-frequency test inputs, which do not produce LTP when stimulated alone, are activated simultaneously with high-frequency conditioning inputs to the same neurons [2,16]. Induction of LTP requires the simultaneous depolarization of the postsynaptic membrane and activation of glutamate receptors of the *N*-methyl-*D*-aspartate (NMDA) subtype [6,10,12,30]. Earliest reports of long-term depression (LTD) of synaptic transmission in the hippocampus described a heterosynaptic LTD for inputs that are inactive [19] or weakly active [17,18] during high-frequency stimulation of a separate converging input. Recently, we described an associative form of LTD in field CA1 that is produced when a low-frequency test input is negatively correlated in time with a second, high-frequency conditioning input [5,27]. In these studies, LTD of synaptic

strength was also produced by activating presynaptic terminals while hyperpolarizing the postsynaptic neuron with somatic current injection. Furthermore, we also found that the NMDA receptor antagonist 2-amino-5-phosphonovaleric acid (AP5) failed to block LTD [27], suggesting the involvement of a non-NMDA subtype of glutamate receptor in the induction of LTD.

Recent studies have uncovered a second messenger-coupled, or metabotropic, subtype of glutamate receptor that stimulates phosphoinositide (PI) hydrolysis in the hippocampus and neocortex [23,25]. The discovery of a selective agonist for this receptor, trans-1-aminocyclopentane-1,3-dicarboxylic acid (ACPD), has allowed this receptor to be distinguished pharmacologically from the ionotropic excitatory amino acid receptors (AMPA, NMDA, kainate) [22]. Dudek and Bear [7] have shown that, in kitten visual cortex, excitatory amino acid-stimulated PI turnover correlates well with the critical period for ocular dominance plasticity. These authors suggested that this mechanism might contribute to use-dependent depression of synaptic strength [3]. Furthermore, it has also been reported that 2-amino-3-phosphonopropionic acid (AP3) is a potent and selective inhibitor of metabotropic receptor-mediated PI turnover [23,24], and mobilization of intracellular Ca²⁺ [13]. In view of these results, we investigated the possible invol-

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vement of metabotropic receptors in LTD, by testing the ability of AP3 to block associative LTD in hippocampal field CA1.

Hippocampal slices (400 μm thick) from adult male Sprague-Dawley rats were prepared by standard methods [26] and incubated in a modified Haas-style interface slice perfusion chamber [11]. Slices were maintained at 34–35°C, pH 7.4, in an artificial cerebrospinal fluid (ACSF) medium bubbled with a 95% O_2 /5% CO_2 mixture. ACSF composition was (mM): NaCl 126, KCl 5, NaH_2PO_4 1.25, MgCl_2 2, CaCl_2 2, NaHCO_3 26, and glucose 10. Extracellular recording electrodes (1–5 $\text{M}\Omega$) were filled with 2 M NaCl, while intracellular electrodes (70–120 $\text{M}\Omega$) were filled with 2 M potassium acetate. Intracellular potentials were amplified through an Axoclamp IIA amplifier with an active bridge circuit in current clamp mode (Axon Instruments). Bipolar stimulating electrodes were made from 50 μm diameter teflon-insulated platinum-iridium wire [26]. D,L-AP3 and D-AP5 (Cambridge Research Biochemicals) were dissolved directly in artificial cerebrospinal fluid immediately before bath application.

Extracellular evoked field potentials were recorded

Fig. 1 2-Amino-3-phosphonopropionic acid (AP3) blocks induction of associative LTD by out of phase stimulation of synapses on hippocampal CA1 pyramidal neurons. *a*: schematic diagram of the recording arrangement in the CA1 pyramidal cell somatic and dendritic layers, and stimulus sites activating two independent groups of Schaffer collateral/commissural afferents (COND and TEST). *b*: schematic diagram of the stimulus paradigms used for extracellular experiments. Conditioning input stimuli were four trains of bursts, given at 10 s intervals, each train consisting of 5 stimuli at 100 Hz, with an interburst interval of 200 ms. Thus, each train lasted 2 s, with 50 pulses per train. When these inputs were IN PHASE, the test single shocks were superimposed on the middle of each burst of the conditioning input. During OUT OF PHASE stimulation, single shocks were timed symmetrically between the bursts [4,21]. *c*: time course of the average percent changes in peak initial slope of the field EPSP (EPSP SLOPE, closed circles) and population spike amplitude (POP SPIKE AMPLITUDE, open circles) of the TEST input evoked potentials. Each point represents the mean \pm S.E.M. of 7 separate experiments in which the same time course of stimulations were given. First, the test and conditioning stimuli were given alone, causing no long-term changes in TEST input responses. 25 μM AP3 was then bath applied, and out of phase stimulation of the TEST plus COND inputs failed to elicit LTD. After washout of AP3, a second identical out of phase stimulation now elicited associative LTD of the test input responses (*, $P < 0.05$, paired *t*-test). *d*: summary of all extracellular experiments showing that AP3 blocks associative LTD. In control medium, out of phase stimulation elicited LTD of both population spikes (solid bars) and EPSP slopes (hatched bars) recorded 30 min after stimulation (*, $P < 0.05$, paired *t*-test compared to prestimulated baselines). In contrast, the same out of phase stimulation in the presence of 20–25 μM AP3 (out of phase in AP3) no longer produced LTD. Note that there were no persistent effects of the application of AP3 itself (AP3 wash in) or the subsequent washout (AP3 washout). (*n*) = number of slices.

from the apical dendritic (population EPSP) and somatic (population spike) layers of CA1 pyramidal neurons, while stimulating electrodes were placed on either side in stratum radiatum, to activate separate converging synaptic inputs (Fig. 1a). One input was selected at random as the test site, while the other served as the conditioning stimulus input pathway. Four low-frequency trains of test stimuli (5 Hz/2 s, every 15 s) were first applied alone (Fig. 1b; TEST) and did not themselves induce long-lasting changes in synaptic strength. The conditioning site was then stimulated alone at high frequency (Fig. 1b; COND: 5 pulse/100 Hz bursts), eliciting homosynaptic LTP of the conditioning pathway (not shown) without persistently altering amplitude of responses to the test input. After these control conditions were met, *D,L*-AP3 (25 μ M) was bath applied (solid bar,

Fig. 1c) for 30 min prior to the application of out of phase stimulation of both pathways together (TEST + COND). This stimulus paradigm, which normally elicited associative LTD of the test pathway, did not do so in the presence of AP3. The slices were then washed in drug-free medium for 45 min, and a second identical out of phase stimulation was applied to both pathways, now eliciting LTD of the test pathway (*, $P < 0.05$ paired t-test compared to prestimulated baselines, $n = 7$ slices).

All experiments involving application of AP3 (20–25 μ M) are summarized in Fig. 1d, showing the percent changes in test pathway evoked EPSP slopes and population spike amplitudes recorded 30 min after each stimulation. Out of phase stimulation of test and conditioning pathways normally elicited significant LTD of test pathway EPSP and population spike, illustrated 30 min post-

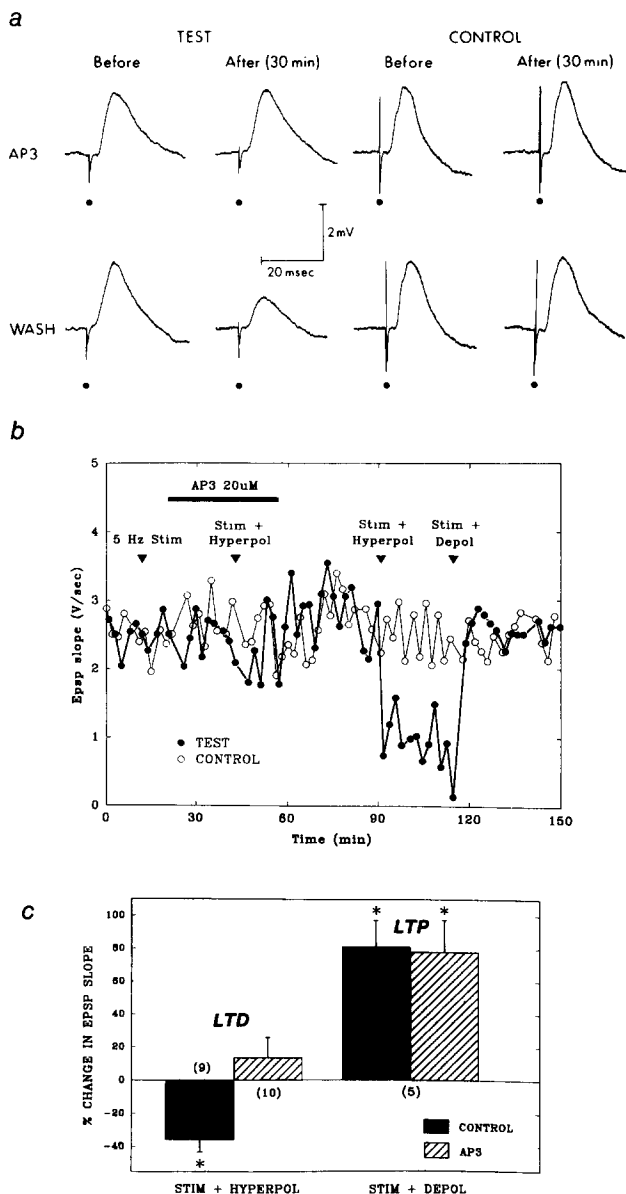


Fig. 2. AP3 blocks LTD induced by pairing postsynaptic hyperpolarization with stimulation of synapses on CA1 pyramidal neurons, but does not block LTP induced by pairing of postsynaptic depolarization with synaptic activation. *a*: intracellular current-clamp records of evoked EPSPs (● denotes stimulus artifact) before and 30 min after pairing hyperpolarizing current injection (constant -0.6 nA current produced a -20 mV hyperpolarization of the soma in the absence of synaptic stimulation) with TEST input synaptic activation (four 5 Hz/2 s trains). In the presence of AP3 (20 μ M), TEST responses showed no persistent changes. After a 60 min drug-free wash, the same hyperpolarization paired with TEST input stimulation were applied a second time, now eliciting homosynaptic LTD of the test pathway. CONTROL synaptic inputs, unstimulated during hyperpolarization, showed no changes in either condition. (RMP = -65 mV, $R_N = 50 \Omega$). *b*: time course of the changes in intracellular EPSP slopes in another experiment. Arrows indicate the times of stimulation of the TEST pathway, while a CA1 pyramidal neuron was at resting potential, hyperpolarized by -20 mV (Hyperpol) or depolarized by $+20$ mV (Depol). Single responses from the test input (filled circles) show that 5 Hz stimulation at rest produced no persistent changes in synaptic strength. In AP3 (20 μ M), 5 Hz stimulation of the TEST input while the cell was hyperpolarized by -20 mV (-0.8 nA injected current) also failed to elicit any long-lasting alterations in EPSPs. Following washout of AP3 for 30 min, the same 5 Hz stimulation was paired with hyperpolarization a second time, and now elicited homosynaptic LTD of the TEST pathway. Finally, this same stimulation in conjunction with $+20$ mV depolarization elicited LTP of the test pathway, indicating that both LTD and LTP mechanisms were functional in this neuron. (RMP = -62 mV, $R_N = 44 M\Omega$). *c*: summary of intracellular experiments showing that AP3 blocks LTD, without impairing LTP, in CA1 pyramidal neurons. In drug-free medium (CONTROL, solid bars), pairing of TEST stimulation (four 5 Hz/2 s trains) with -20 mV hyperpolarization (Stim + Hyperpol) elicited LTD (*, $P < 0.05$ compared to prestimulation baselines) of maximal EPSP slope measured 30 min after stimulation. In AP3 (20 μ M, hatched bars), the same pairing of stimulation with hyperpolarization failed to induce LTD. In contrast, pairing of this same TEST stimulation with $+20$ mV depolarization (Stim + Depol) elicited robust LTP in both CONTROL and AP3. (Mean \pm S.E.M., (n) = number of cells.)

stimulation (OUT OF PHASE, $*P < 0.05$ paired *t*-test, (n) = number of slices). In contrast, a 30 min bath application of AP3 prior to the same out of phase stimulation completely blocked LTD in CA1 pyramidal neurons (OUT OF PHASE IN AP3), without producing any significant changes in EPSP slope or population spike amplitude by itself (AP3 WASH IN).

In previous studies, we have shown that LTD can be induced by pairing hyperpolarizing current injected into the soma with presynaptic activation [27]; Similar results have been reported in visual cortical neurons [1]. Therefore, we also examined the ability of AP3 to block LTD induced by such pairing in intracellular recordings from CA1 pyramidal neurons. Fig. 2a illustrates typical EPSPs recorded intracellularly in CA1 pyramidal neurons and evoked by stimulating on the Schaffer collateral (TEST) or subicular (CONTROL) sides in the apical dendritic layer (stratum radiatum). AP3 (20 μ M) was bath applied for 30 min prior to stimulation, and had no significant effect on resting membrane potential, input resistance, evoked synaptic potentials or threshold for action potential firing (not shown). The soma was then hyperpolarized -20 mV by intracellular current injection through the recording electrode, and the test pathway stimulated with 4 low-frequency trains (5 Hz/2 s, every 15 s). Current injection was then turned off, and the evoked EPSPs in each pathway tested by alternating single shocks each 30 s. Fig. 2a shows evoked responses immediately before, and 30 min after, test pathway stimulation paired with hyperpolarization. In the presence of AP3, stimulation did not produce persistent alterations in evoked potentials in either test or control pathways. Thereafter, the slice was washed in drug-free medium for 60 min and the same stimulation and hyperpolarizing current was applied a second time, which now elicited LTD, an approximately 50% reduction in the test pathway evoked EPSP 30 min after the second stimulation.

Fig. 2b shows the time course of a representative intracellular experiment, graphing the maximum initial slope (V/s) of the EPSP evoked by test (closed circles), and control (open circles), pathway stimulation. First, test pathway stimulation (5 Hz/2 s \times 4) was applied alone, and had no effect on synaptic strength. Then, AP3 (20 μ M, bar) was bath applied for 30 min prior to another series of four 5 Hz trains given while hyperpolarizing by -20 mV. This pairing failed to elicit LTD of test pathway synaptic strength, after which AP3 was washed out for an additional 30 min and the same stimulation paired with hyperpolarization applied a second time. This stimulation paradigm now elicited marked homosynaptic LTD of the test pathway, persisting for 30 min. At this time, another series of four low-frequency trains was

again applied to the test pathway, this time while depolarizing the neuron by $+20$ mV, eliciting associative LTP of this pathway, which returned evoked responses to their original amplitude. Intracellular experiments are summarized in Fig. 2c, illustrating the percent change in EPSP slope elicited by pairing of synaptic stimulation with hyperpolarization (LTD) or with depolarization (LTP), in the absence and presence of 20 μ M AP3. AP3 completely prevented the induction of LTD, measured 30 min after stimulation ($n = 10$). Then, AP3 was washed out and the same stimulation given a second time, which now led to significant LTD of the test pathway ($*, P < 0.05$, paired *t*-test compared to pre-stimulus baselines, $n = 9$). In contrast, LTP was unaffected by the same concentration of AP3 (Fig. 2c; LTP, $n = 5$).

Our observations that AP3 had no effect on induction of associative LTP, induced either by in phase stimuli or pairing depolarization with synaptic activation, suggests that AP3 does not significantly block NMDA receptors. We further verified that NMDA receptors do not play a direct role in LTD induction by applying the NMDA antagonist AP5 during the pairing of intracellular hyperpolarization with presynaptic stimulation. The experimental paradigm was identical to previous studies, except that D-AP5 (20 μ M) was bath applied. LTD was elicited in the presence of AP5 ($-52.6 \pm 8.8\%$, $n = 6$), and did not differ from that observed in control drug-free experiments. In our initial studies, we also found that AP5, which blocked LTP induction at synapses on apical dendrites of CA1 pyramidal neurons, had no effect on LTD of these same synapses [27]. Our present findings supply the first evidence implicating a specific non-NMDA glutamate receptor, the metabotropic (ACPD) glutamate receptor, in the induction of LTD in the hippocampus.

The evidence for homosynaptic depression in neocortex is consistent with the characteristics of LTD that we have found in the hippocampus. Studies in neocortical slices [1,8] suggest that there is a narrow window of membrane potentials for eliciting depression: there is no long-term change in synaptic strength if the membrane potential is below the threshold for depression, and LTP occurs if the membrane potential is above a second threshold. However, at present, mechanisms linking the voltage sensitivity of LTD to the involvement of metabotropic receptors are not clear. Furthermore, another common feature underlying hippocampal and neocortical LTD is that it results when a synapse is active and when its use is uncorrelated with activity at other synapses on the same neuron. The signal for lack of correlation might be the changes in calcium levels in dendritic spines. One such mechanism, absence of calcium influx through NMDA receptor channels during presy-

naptic activity, is apparently not sufficient to induce LTD or reverse LTP [9]. But the extent, spatial location and time course of rise in intracellular calcium concentration may nonetheless be important, since activation of metabotropic receptors can trigger the release of calcium from intracellular stores [13,20].

Recent evidence indicates that in kitten striate cortex, the age-dependent coupling of excitatory amino acid-stimulated PI hydrolysis correlates with postnatal changes in cortical susceptibility to visual deprivation [7]. Furthermore, AP3 treatment in 2-month-old kittens can block the electrophysiological effects of monocular deprivation on the striate cortex contralateral to the deprived eye [3]. Thus, in the visual cortex, experience-dependent reductions in synaptic strength may be dependent on metabotropic receptor activation leading to LTD of deprived eye synapses. In the cerebellum, LTD at parallel fiber-Purkinje cell synapses occurs when parallel fiber inputs are paired with climbing fiber inputs [14]. Recent studies suggest that, in this form of LTD, AMPA-specific glutamate receptors mediating parallel fiber-Purkinje cell responses are desensitized through a process which involves co-activation of metabotropic ACPD receptors [15]. In agreement with these recent findings, our results suggest that activation of metabotropic receptors may be required for induction of LTD at hippocampal synapses. However, this interpretation is based on assumptions of the specificity and mechanism of action of AP3 that are still uncertain. There is evidence suggesting variations between the pharmacology of electrophysiological effects and other biochemical responses elicited by metabotropic receptor activation [28,29]. Further studies will be required to determine the biochemical links between metabotropic receptor stimulation and the induction of LTD.

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