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Long-term synaptic transformation of hippocampal CA1 γ-aminobutyric acid synapses and the effect of anandamide

(long-term potentiation/Cl^- channels/cannabinoid receptors)

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ABSTRACT **Evidence is presented for a distinctive type of hippocampal synaptic modification [previously described for a molluscan γ-aminobutyric acid (GABA) synapse after paired pre- and postsynaptic excitation]: transformation of GABA-mediated synaptic inhibition into synaptic excitation. This transformation persists with no further paired stimulation for 60 min or longer and is termed long-term transformation. Long-term transformation is shown to contribute to pairing-induced long-term potentiation but not to long-term potentiation induced by presynaptic stimulation alone. Further support for such mechanistic divergence is provided by pharmacologic effects on long-term transformation as well as these two forms of long-term potentiation by Cl^- channel blockers, glutamate and GABA antagonists, as well as the endogenous cannabinoid ligand anandamide.**

Enhanced hippocampal synaptic excitation or long-term potentiation (LTP) after high-frequency stimulation of the presynaptic Schaffer collaterals has been the subject of extensive analyses (1, 2). Most studies have not clearly distinguished between mechanisms responsible for LTP induced by tetanic stimulation alone (see ref. 1) and those responsible for LTP induced by pairing—i.e., after stimulation of presynaptic fibers paired with postsynaptic depolarization of the recorded (3) pyramidal cells. This report demonstrates that when postsynaptic depolarization is paired with presynaptic tetani, γ-aminobutyric acid-mediated (GABAergic) synaptic inhibition is transformed into GABAergic excitation. This synaptic transformation persists with no further paired stimulation for 60 min or longer and is termed long-term transformation (LTT). This synaptic LTT is shown to contribute significantly to pairing-induced LTP but not to tetanus-induced (i.e., not paired with depolarization) LTP. The same synaptic transformation was previously identified for the GABA synapses of the visual–vestibular sensory cells of the snail *Hermissenda* (4).

MATERIALS AND METHODS

The slicing procedure has been described (5). In brief, recordings were obtained in 400-μm-thick transverse hippocampal slices of adult (150 g) male Sprague–Dawley rats. Slices were maintained at 34–35°C in a submersion chamber perfused with artificial cerebrospinal fluid (126 mM NaCl/3.0 mM KCl/1.25 mM NaH_2PO_4/24 mM $NaHCO_3$/2.0 mM $MgSO_4$/2.0 mM $CaCl_2$/10 mM glucose). The flow rate was adjusted to 1.5 $ml \cdot min^{-1}$ and was bubbled with 95% O_2/5% CO_2. Intracellular recordings were obtained with micropipettes filled with 1 M KAc/1 mM KCl and with a resistance of 60–80 MΩ. Intracellular recordings from the CA1 neurons were amplified with an Axoclamp 2A amplifier. Data were digitized at 1–10 KHz and stored in magnetic tape for later analysis using the PCLAMP computer program. Bipolar tungsten electrodes were placed at the stratum radiatum to stimulate the Schaeffer collaterals or very close to the intracellular electrode to directly stimulate the basket interneurons at the pyramidal layer.

RESULTS

Exogenous GABA application to CA1 pyramidal cell somata (Fig. 1 *A* and *B*) elicited a hyperpolarizing response. It was often possible to discriminate two components within the GABA-elicited hyperpolarization. An initial fast-hyperpolarizing component (Fig. 2*A1 Left*, red trace) reversed at about −70 mV (±5) (Fig. 2 *A1* and *C*). This fast component was blocked by furosemide (0.8 mM), $GABA_A$ receptor-mediated Cl^- pump blocker (7) and bicuculline methiodide (BMI), a $GABA_A$ receptor blocker (Fig. 2 *A2*). These observations are consistent with previous findings (6, 8, 9), and can be explained by $GABA_A$ receptor-mediated opening of Cl^- channels (10, 11). A later, slower, and somewhat variable hyperpolarizing component (Fig. 2*A1*, red trace) was blocked by phaclofen (Fig. 2 *A2*) but not by furosemide and can be explained by $GABA_B$ receptor-mediated opening of K^+ channels (12, 13). The cell-membrane voltage changes to repetitive hyperpolarizing current pulses during the GABA responses were reduced, indicating that GABA applications caused a net increase in membrane conductance.

GABA application paired with postsynaptic depolarization (Fig. 1*B2*) was initially followed by a transient depression of the hyperpolarizing response elicited by GABA alone (12–14). Subsequently, a depolarizing component appeared 30 sec after pairings. Further responses to GABA alone, elicited 10 min after the paired stimulation, were purely depolarizing and remained so with no further paired stimulation for >60 min (Figs. 1 *B2 Right*, green trace, and 2 *B1* and *D*). This long-lasting change of a hyperpolarizing response into a depolarizing response, we called LTT. The reversal potential of the depolarizing GABA responses after LTT (Fig. 2*B1*) ranged from −35 to −45 mV (Fig. 2*C*). The transformed GABA response was only slightly reduced or not affected at all by the $GABA_B$ antagonist phaclofen (Fig. 2*B2*). By contrast, the Cl^- pump antagonist furosemide or the $GABA_A$ antagonist BMI, both in the absence of phaclofen, eliminated (also with phaclofen as in Fig. 2*B2*) the transformed responses (n = 22). Tetrodotoxin (1 μM), a Na^+-channel blocker; ω-conotoxin GVIA (a somewhat selective N-type Ca^{2+}-channel blocker); or

Abbreviations: GABA, γ-aminobutyric acid; $GABA_A$ and $GABA_B$, GABA type A and type B, respectively; GABAergic, GABA-mediated; LTP, long-term potentiation; LTT, long-term transformation; CNQX, 6-cyano-2,3-dihydroxy-7-nitroquinoxaline; epsp and ipsp, excitatory and inhibitory postsynaptic potential(s), respectively; AP5, DL-amino-5-phosphonovalerate.

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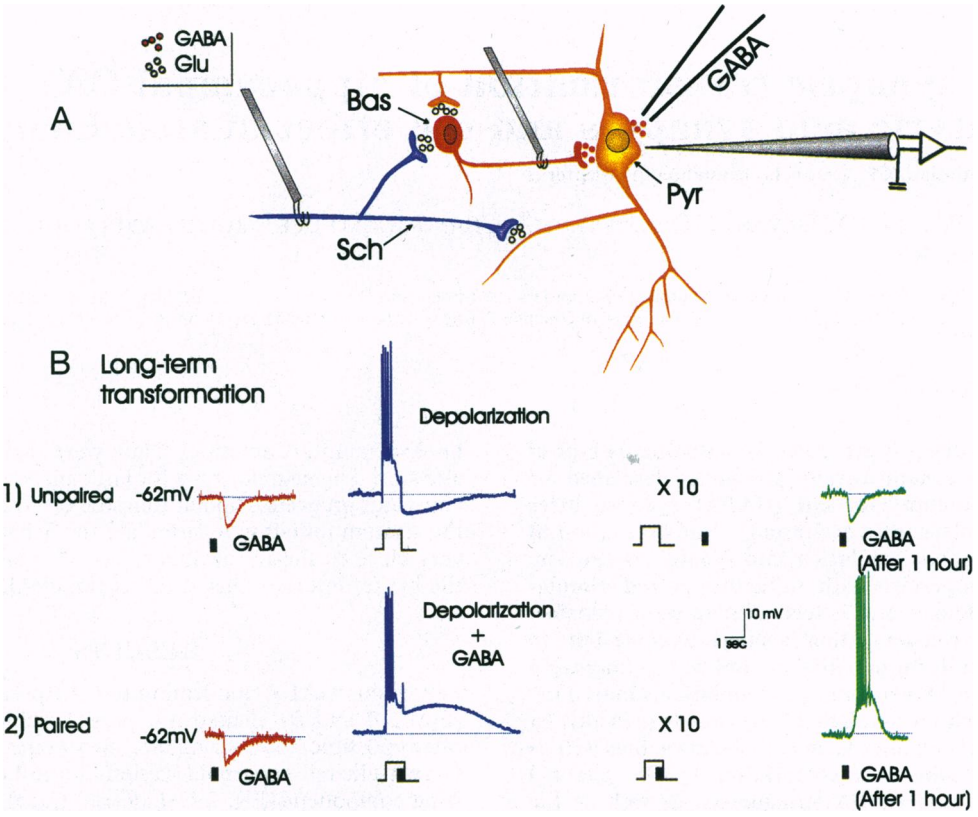

FIG. 1. Long-term transformation of somatic hyperpolarization in response to exogenous GABA application. (*A*) Excitatory and inhibitory inputs to the CA1 pyramidal (Pyr) neurons in rat hippocampus. Excitatory input from Schaffer collaterals (Sch) to CA1 dendrites is mediated by glutamate (Glu). Inhibitory inputs are mediated by GABA released from three spatially segregated types of basket cell (Bas) interneurons (9), each specifically innervating dendrites, soma, or axon of the postsynaptic CA1 neuron. Only basket-to-soma interneurons are shown here to illustrate recording conditions. (*B*) GABA (indicated by black bar) was applied with a 30-msec 20-psi (1 psi = 6.9 kPa) pressure pulse through an ejection pipette [containing 1 mM GABA dissolved in artificial cerebrospinal fluid] near the CA1 pyramidal soma and elicited a pure hyperpolarizing response (red trace) with repeated applications at 2-min intervals. (*1*) After test applications of GABA alone that elicited hyperpolarizing responses (*Left*, red trace), depolarizing steps alone elicited a train of action potentials followed by an afterhyperpolarization (*Middle*, purple trace). Repeated alternation (10 times at 5-sec intervals) of depolarizing steps with GABA application alone (unpaired stimulation) did not change the responses to GABA puffs (*Right*, green trace). (*2*) Similar GABA puffs were delivered immediately before termination of a 1-sec depolarizing current step, as indicated by an open bar (paired stimulation). Paired stimulation was repeated 10 times at 5-sec intervals. The purple trace (*Middle*) shows a typical voltage response during one such pairing. Current intensity was sufficient (1.0–2.0 nA) to elicit at least five action potentials that were interrupted by the onset of the GABA puff. Typically, paired stimulation produced a late, prolonged depolarization after the train of action potentials (*Middle*, purple trace). After 10 pairings, GABA alone elicited a transient and sometimes reversible depression of the fast hyperpolarizing response together with a depolarizing wave (data not shown). Within 10 min of such a pairing, the responses to GABA alone had become transformed into depolarizations, which often elicited action potentials (*Right*, green trace).

nifedipine, an L-type Ca^{2+}-channel blocker, did not prevent LTT induction, suggesting that a presynaptic mechanism is not involved (data not shown). The transformed response was also associated with an increased membrane conductance. Unpaired stimulation (i.e., GABA application alternating with postsynaptic depolarization), repetitive stimulation with GABA application alone, or depolarizing steps alone failed to produce LTT (Fig. 1*B1*). To produce LTT, it was also necessary for the initial GABA-elicited response to be purely hyperpolarizing. Biphasic GABA responses (e.g., elicited with high—i.e., > 2 mM—GABA concentrations) were blocked by BMI and did not become transformed after pairings.

LTT was consistently observable in recordings from the CA1 soma. Dendritic application of GABA consistently produced biphasic responses with a large depolarizing component. Pairings of GABA application to the dendrites with depolarizing steps delivered to the soma failed to produce any significant change of the GABAergic response (data not shown). Furosemide also had no effect on the dendritic response to GABA (4). Bicuculline, however, blocked the dendritic response to GABA.

Removal of external Na^+ did not reduce the transformed (i.e., depolarizing) GABA response, nor did changes of external Na^+ significantly change the reversal potential of the GABA response ($n = 6$). Similarly, removal of external Ca^{2+} or multiple elevations of external Ca^{2+} had no significant effect ($n = 6$) on either the magnitude or reversal potential of the transformed (depolarizing) GABA response. Thus, the transformed GABA depolarizing response does not result from increased conductance to Na^+ and/or Ca^{2+} as is the case for a classical epsp, such as in response to glutamate. Although changes of external K^+ did (predictably) shift the membrane potential, as well as the reversal potential for the late phase of the GABA-elicited hyperpolarization, no such effect was seen for the GABA-elicited depolarization ($n = 6$). Changes of external Cl^- concentrations shifted the reversal potential of the early phase of the GABA-elicited hyperpolarization (as previously described) but had only slight and variable effect on the GABA-elicited soma depolarization ($n = 6$).

These results, considered together with the previously observed reversal potentials for the early and late GABA-elicited hyperpolarization, suggest that the GABA-elicited depolarization depends on a still poorly understood mixed conductance mechanism (e.g., involving Cl^-, HCO_3^-, and possibly other ions). Such a mixed conductance change has been suggested to be effected by a Cl^- exchange pump as previously considered

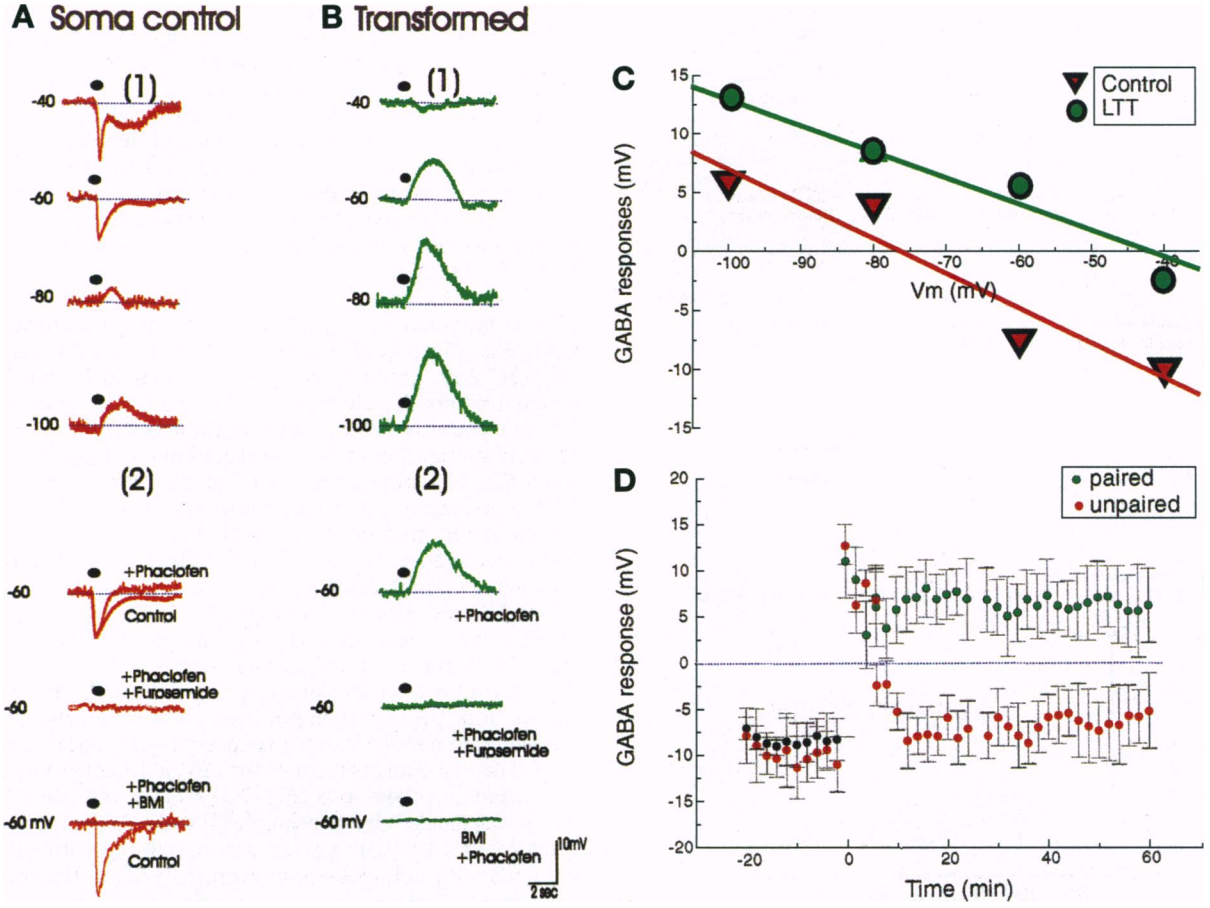

FIG. 2. Characteristics of LTT of postsynaptic GABAergic responses. (*A*) (*1*) GABA (application time indicated by black dots) elicits a fast, early hyperpolarizing response (reversing at −70 mV), followed by a slower, but prolonged, hyperpolarization (reversing at −80 mV). (*2*) Addition of phaclofen to the bath (30 μM) eliminates the late response. The fast response is blocked by subsequent addition to the bath of 0.6 mM furosemide or 50 μM BMI. (*B*) (*1*) The transformed responses to GABA (same cell as in *A*) are shown to reverse between −35 and −45 mV. (*2*) Phaclofen (30 μM) does not block, and sometimes enhances, the transformed response. Furosemide or BMI (in the presence of phaclofen) blocked the transformed responses ($n = 6$). BMI application was not preceded by furosemide. Bottom trace was from a different cell than for upper two traces. (*C*) Reversal potentials of the responses shown in *A*. The early, GABA-mediated, fast-hyperpolarizing responses reversed at about −70 mV (red triangles). The transformed responses (green circles) reversed at −43 mV. (*D*) LTT of the soma GABA responses recorded at resting membrane potential was long-lasting. GABA puffs paired with postsynaptic depolarization transformed an initially hyperpolarizing response (−8.3 mV ± 3.2, SD, $n = 20$) into depolarizing responses (6.2 ± 4.1 mV, SD, $n = 20$, $P < 0.005$, t test). Unpaired GABA puffs and postsynaptic depolarization did not change the hyperpolarizing response.

in other types of GABA-induced depolarizing responses (11, 14–17) and that would be expected to be blocked by furosemide (see above, Fig. 2*B2*).

Because phaclofen blocked the late GABA-elicited hyperpolarization, but not the GABA-elicited soma depolarization, the latter does not seem to involve GABA type B receptor-mediated depolarization. Because both bicuculline and furosemide did block the GABA-elicited soma depolarization, this response does appear to depend on $GABA_A$ receptor activation.

Tetanic stimulation (see Fig. 3) of the presynaptic Schaffer collaterals caused a transient enhancement, also called post-tetanic potentiation (data not shown), of a typical glutamate-mediated epsp (Fig. 3*A Left*, green trace) recorded intracellularly from a hippocampal CA1 pyramidal cell. This initial enhancement was followed by a long-lasting enhancement or LTP of the epsp beginning 10 min after stimulation and lasting >60 min (in 20 of 25 cells) (Fig. 3*A Middle*, red trace). Increasing the number of trains (7 to 10) induced LTP (9 of 10 cells) without increasing magnitude and duration of synaptic potentiation. Pairing-induced LTP was produced (Fig. 3*B*) by pairing high-frequency stimulation (7 trains) of the Schaffer collaterals with postsynaptic depolarization to 0 mV (produced by positive current injection through the intracellular microelectrode) in 20 of 20 cells.

The epsp recorded from the CA1 pyramidal cell before LTP (Fig. 3*A Left*) could be blocked (data not shown) by the α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) receptor antagonist 6-cyano-2,3-dihydroxy-7-nitroquinoxaline (CNQX) (100 μM) together with the *N*-methyl-D-aspartate receptor antagonist DL-amino-5-phosphonovalerate (AP5) (50 μM) (18). Ten minutes after pharmacological blockade of the glutamate-induced epsp, a fast ipsp is unmasked. This ipsp has been reported to be mediated by $GABA_A$ receptors localized at the soma (7). Furosemide, an antagonist of the Cl^- pump blocks this soma ipsp elicited by Schaeffer collateral stimulation but does not block the ipsp that involves the activation of $GABA_A$ receptors localized in the dendrites. Furosemide enhanced the epsp before its blockade with CNQX and AP5 (data not shown).

Addition of furosemide or BMI 10 min after LTP induction with the tetanic stimulation-alone protocol caused some additional enhancement of the already potentiated epsp (Fig. 3*A*, *Right*, black trace). This enhancement is consistent with previous findings suggesting that maximal LTP is achieved in the presence of $GABA_A$ blockers such as BMI or picrotoxin (1). This effect can be interpreted as a blockade of the underlying

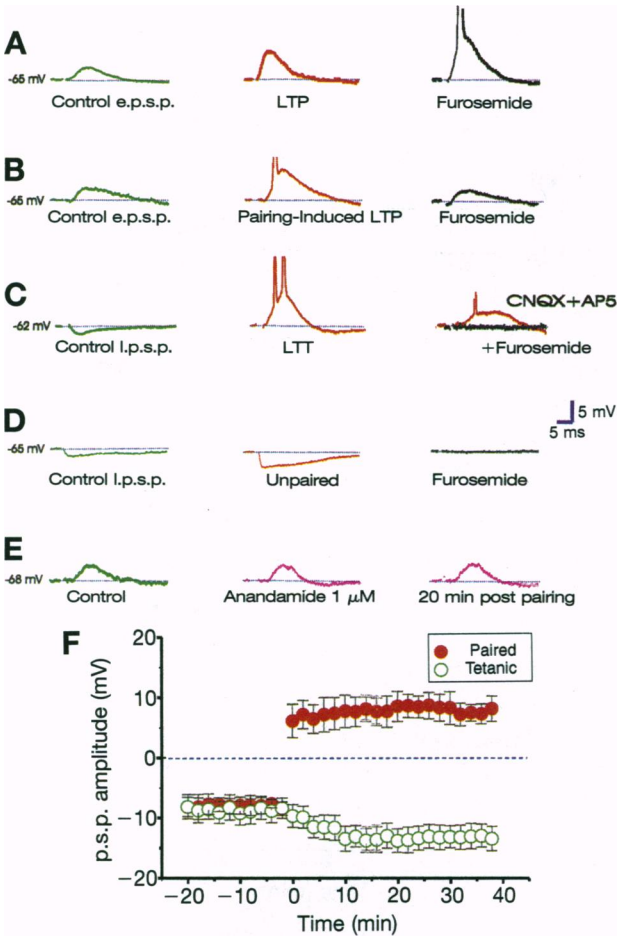

FIG. 3. Pharmacological differences among LTP, pairing-induced LTP, and LTT. (*A*) epsp (*Left*, green trace) elicited by a 10-μsec presynaptic shock to the Schaeffer collaterals (control) was potentiated (*Middle*, red trace) 20 min after a series of high-frequency trains of stimulation (10 300-msec, 100-Hz trains, every 5 sec). Test stimulus intensity for the epsp was adjusted to half that necessary to elicit a maximum response. Ten to 20 min after addition of 600 μM furosemide to the perfusate, a further enhancement of the potentiated epsp was observable (*Right*, black trace). (*B*) Control epsp (*Left*, green trace) was also potentiated (*Middle*, red trace) 20 min after a series of 10 postsynaptic-depolarizing 1-sec steps paired with a coterminating, 300-msec, presynaptic tetanus. Depolarizing step amplitudes were adjusted to elicit at least five action potentials. Furosemide (600 μM) perfusion eliminated the enhancement of the epsp after pairing-induced LTP (*Right*, black trace). (*C*) Direct stimulation of basket cells innervating the CA1 soma was effected by placing the stimulating electrode at the stratum pyramidale, very close to the recording electrode, in normal artificial cerebrospinal fluid. Such stimulation, 10-μsec test pulses, elicited a monosynaptic ipsp (*Left*, green trace) even at high stimulus intensities. Ten minutes after a series of 10 presynaptic tetani paired with postsynaptic depolarizing steps (protocol as for pairing-induced LTP) test pulses elicited a fast depolarizing epsp (LTT), followed by a later ipsp (see also *F*). CNQX plus AP5 added after LTT induction reduced, but did not eliminate, LTT. Perfusion with furosemide, in addition to CNQX plus AP5, blocked the transformed response as shown (*Right*, black trace). (*D*) ipsp elicited as in *C* (*Left*, green trace) were slightly enhanced (see also *F*) and not transformed by the same postsynaptic depolarizations alternating with presynaptic tetani (*Middle*, red trace). Furosemide perfusion eliminated the untransformed ipsp. (*E*) CA1 amplitudes of postsynaptic potentials (*Left*, green trace) elicited as in *B* were not affected by anandamide (*Middle*, red trace) which prevented pairing-induced LTP (*Right*, red trace). Anandamide (1 μM) was added in the presence of bovine serum albumin at 5 mg/ml. All recording traces were filtered at 3 KHz, except for *A*, which was filtered at 1 KHz. (*F*) Graphical depiction of additional replications ($n = 5$) of protocol in *C* (upper curve) and protocol in *D* but with presynaptic tetani alone ($n = 4$). Error bars indicate ±SD. p.s.p., Postsynaptic potential.

GABAergic ipsp. Furosemide had an opposite effect on pairing-induced LTP. Furosemide caused a reduction of ≈50% of the epsp potentiation in 12 cells or a complete elimination of the potentiation in 8 out of 20 cells (Fig. 3*B Right*, black trace). This can be explained as furosemide blocking an *excitatory* $GABA_A$-mediated depolarization (Fig. 1*B2*), and it suggests that an underlying LTT contributes to the expression of pairing-induced LTP. Furthermore the presence of AP5 (50 μM) and CNQX (100 μM) in the external bathing medium reduced, but did not eliminate, the pairing-induced LTP (20/20 cells).

Additional support for the role of LTT in pairing-induced LTP is provided by effects on isolated ipsps recorded from CA1 cells (Fig. 3 *C*, *D*, and *F*). Recordings of the isolated ipsp (Fig. 3*C Left*, green trace) can be obtained by placing the stimulating bipolar electrode in the stratum pyramidale (Fig. 1*A*), as close as possible to the recording electrode. This results in direct stimulation of the basket cells that synapse on the CA1 soma (3). The stimulation protocol used for pairing-induced LTP transformed the isolated ipsp into an epsp 10 min after the induction protocol in 10 of 10 cells (Fig. 3*C Middle*, red trace). AP5 (50 μM) together with CNQX (100 μM) reduced, but did not eliminate, the pairing-induced LTT (5/5 cells) (Fig. 3*C Right*, red trace). Subsequent addition of furosemide blocked the transformed (LTT) synaptic potential (Fig. 3*C, Right*, black trace), an effect consistent with GABA-mediated depolarization contributing to pairing-induced LTP. Unpaired stimulation (i.e., presynaptic stimulation alternating with postsynaptic depolarization) or presynaptic tetani alone not only failed to transform the ipsp (10 of 10 cells) but caused enhancement of the ipsp (Fig. 3 *D Middle*, red trace and *F*), which was blocked by furosemide (Fig. 3*D Right*, black trace). Identical results (with paired and unpaired protocols) were obtained with only potassium acetate (3 M) in the recording electrodes.

High levels of the brain cannabinoid receptor (19), as well as an enzyme capable of synthesizing anandamide (20), an endogenous ligand for the cannabinoid receptor (21), are present in the hippocampus. Because anandamide can interact with muscimol-induced GABAergic responses (22), we tested the possibility that LTT of GABA synapses and pairing-induced LTP might be influenced by the endogenous cannabinoid receptor agonist anandamide.

Anandamide (1 μM) invariably prevented (Fig. 4*A*, trace 3) the development of LTT in the soma but did not affect hyperpolarizing responses to exogenous GABA soma application alone (Fig. 4*A*, trace 2). At higher concentrations (10 μM), or with prolonged exposure (e.g., >1 hr with 1 μM anandamide), the duration of the late soma hyperpolarization was prolonged in most cases (7 out of 10 cells, Fig. 4*A*, trace 5). Anandamide blockade of LTT at 1 μM was reversible 1 hr after washout. LTT was thus inducible after this washout of anandamide [Fig. 4*A*, trace 4 (green)], whereas prolonged exposure to higher (>1 μM) concentrations of anandamide caused irreversible effects. Perfusion of 1 μM anandamide prevented pairing-induced LTP (Figs. 3*E*, purple trace and 4*C*), but it did not prevent the transient potentiation known as posttetanic potentiation (Fig. 4*C*). Other experiments ($n = 6$) indicate that anandamide (1 μM) had no consistent effects on LTP produced by presynaptic stimulation alone. Consistent prevention by anandamide of both LTT (Fig. 4*B*) and pairing-induced LTP (Fig. 4*C*) is additional evidence that these two long-term synaptic modifications share some common mechanism(s). Anandamide alone, as well as pairings in the presence of 1 μM anandamide, had no noticeable effect on the dendritic GABA responses.

DISCUSSION

The long-terminal synaptic transformation (4) recently observed in the visual–vestibular network of a mollusc (in

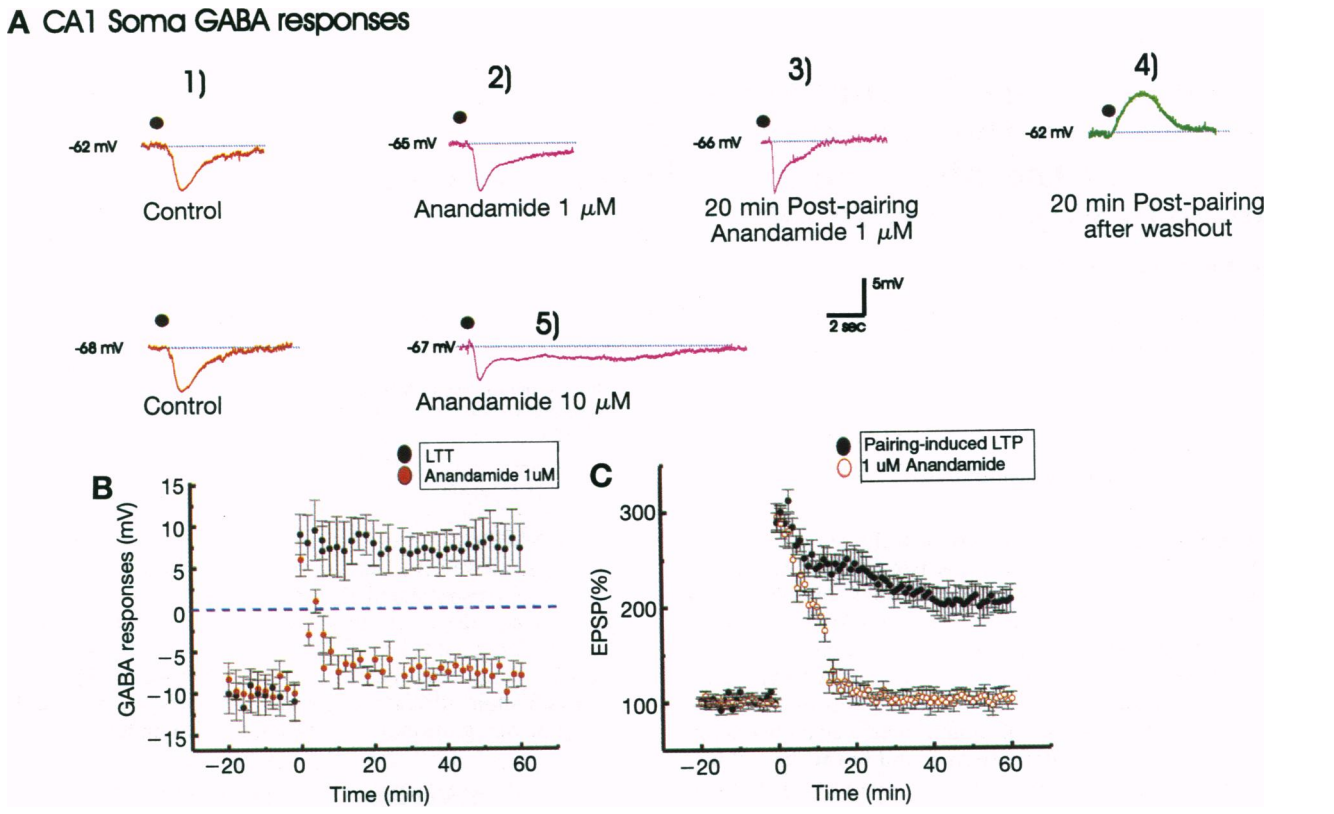

FIG. 4. Anandamide prevents LTT and pairing-induced LTP. (*A*) Postsynaptic responses to applied GABA (trace 1) were not changed after 20 min of 1 μM anandamide (trace 2); 1 μM anandamide blocked LTT after pairings of GABA and depolarization as shown here 20 min after pairings (trace 3); 60 min after continuous washout with perfusate containing bovine serum albumin alone—i.e., without anandamide—new pairings induced LTT (trace 4); 10 μM anandamide did not change the fast GABA response amplitude, but it irreversibly prolonged the late component (trace 5). (*B*) Anandamide (1 μM) prevented LTT in four of four cells (black dots) but did not prevent the transient appearance of a depolarizing response within 10 min after pairings. (*C*) Anandamide (1 μM) prevented (red open dots) pairing-induced LTP (black dots) but not posttetanic potentiation. The plots illustrate the normalized percentage change of epsp after paired stimulation. Test responses were elicited as shown in Fig. 3 (n = 10). Error bars indicate ±SD.

response to behaviorally relevant stimuli) is shown here in the rat hippocampus. Pairing of pre- and postsynaptic excitation transformed ipsps elicited by basket cell stimulation into epsps (Fig. 3*C*) for at least 60 min. Moreover, exogenous GABA when paired with postsynaptic depolarization also produced LTT. LTT contributes to pairing-induced LTP but differs from changes previously observed for GABAergic synapses after LTP (23–25). Both LTT and pairing-induced LTP were prevented by 1 μM anandamide.

Receptor-mediated anandamide/cannabinoid responses include inhibition of adenylate cyclase (26) and N-type Ca^{2+} channels (27). The inhibition by anandamide of LTT and associatively induced LTP is consistent with one or more of these receptor-mediated inhibitory effects. A possible physiologic role for anandamide in behavioral learning, therefore, is to inhibit storage of less relevant sensed information that might obscure storage of information that is at the focus of attention.

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