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Neuroscience Letters 238 (1997) 119–122

Neuroscience Letters

Contributions of AMPA and $GABA_A$ receptors to the induction of NMDAR-dependent LTP in CA1

C. Debray, D. Diabira, J.L. Gaiarsa, Y. Ben-Ari, H. Gozlan*

Institut National de la Santé et de la Recherche Médicale U-29, 123 Bld de Port-Royal, 75014 Paris, France

Received 30 June 1997; received in revised form 12 November 1997; accepted 12 November 1997

Abstract

The contributions of (±)-α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) and γ-aminobutyric acid ($GABA_A$) receptors in the induction of long-term potentiation (LTP) have been studied in the CA1 region of the rat hippocampus. The results suggest that: (1) in physiological conditions, AMPARs are necessary for the induction of *N*-methyl-D-aspartate receptor (NMDAR)-dependent LTP since LTP cannot be elicited in the presence of the AMPAR antagonist, 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX). Although a NMDAR-dependent LTP occurs in the presence of a $GABA_A$ antagonist and high concentrations of divalents cations, blockade of AMPARs leads to a voltage-dependent calcium channels (VDCC)-dependent LTP since its induction is blocked by nifedipine and not by APV. (2) The bicarbonate-induced $GABA_A$ receptor-mediated depolarizing response is not necessary in the induction of NMDAR-dependent or VDCC-dependent LTP since induction of these two types of LTP were not blocked by acetazolamide or in a nominally bicarbonate-free solution. © 1997 Elsevier Science Ireland Ltd.

Keywords: AMPA receptors; *N*-Methyl-D-aspartate receptors; γ-Aminobutyric acid receptors; Voltage-dependent calcium channels; Long-term potentiation induction; CA1; 6-Cyano-7-nitroquinoxaline-2,3-dione; Acetazolamide

In the CA1 area of the hippocampus, a postsynaptic depolarization is required to remove the magnesium block of *N*-methyl-D-aspartate receptors (NMDARs) and to induce the NMDAR-dependent long-term potentiation (LTP) [12]. While not directly demonstrated, the activation of AMPA receptors (AMPARs) during high frequency stimulation (HFS) is thought to provide this source of depolarization. However, there is a surprising shortage of experimental work using AMPAR antagonists that would directly address this point. In fact, it has been reported that the blockade of AMPARs during the tetanus does not prevent LTP induction [9,12]. Furthermore, if AMPARs are not necessary for LTP induction, other sources must provide the postsynaptic depolarization required to remove the voltage-dependent magnesium block of NMDARs. γ-Aminobutyric acid receptors ($GABA_A$Rs) have been suggested to be able to play such a role under certain conditions [5,14].

The present study was centered on three questions: (1) are AMPARs necessary for the induction of NMDAR-dependent LTP under physiological conditions?; (2) what is the contribution of the bicarbonate-induced $GABA_A$R-mediated depolarizing response to the induction process; (3) could $GABA_A$Rs activation during HFS represent an alternative way for the activation of NMDARs when AMPARs are blocked?

Experiments were performed in hippocampal slices obtained from male Wistar rats (3–5 weeks old) as previously described [3]. Slices were placed in a fully submerged chamber and superfused at 30°C, with an artificial cerebrospinal fluid (ACSF) containing (in mM): $NaHCO_3$ (25), NaCl (126), KCl (3.5), $MgCl_2$ (1.3), NaH_2PO_4 (1.2), $CaCl_2$ (2), and glucose (11) and gassed with 95% O_2 and 5% CO_2, pH 7.4. Experiments were also performed in ACSF containing higher concentrations (4 mM) of Ca^{2+} and Mg^{2+}, hereafter termed ACSF4/4. Field excitatory postsynaptic potentials (fEPSPs) were evoked by electrical stimulation of Schaffer collateral fibers (0.033 Hz, 30 μs duration) and recorded in the stratum radiatum (≈ 200 μm from the pyramidal layer) with a glass microelectrode filled with 3 M

* Corresponding author. Tel.: +33 1 53737932; fax: +1 33 1 46341656; e-mail: gozlan@cochin.inserm.fr

0304-3940/97/$17.00
PII S0304-3940(97)00865-3

NaCl. Initial slopes of fEPSPs were measured during the first ms after the afferent volley. LTP, evoked by two trains (100 Hz, 1 s duration, 20 s intertetanus interval), was defined as an increase of the slope of the fEPSP greater than 20% that remains stable for at least 30 min. Intracellular recording were obtained from CA1 pyramidal cells using potassium methylsulfate-filled electrode (2 M). Only cells displaying a stable membrane potential around −60 mV were selected. Data are the mean ± SEM. Student's paired or impaired *t*-tests were used in the statistical analysis.

A HFS applied in presence of 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX; 10 μM) once the fEPSP was fully blocked did not generate LTP upon wash-out of CNQX (+6 ± 7%, $n = 12$) (Fig. 1A). In the same conditions, increasing the number of tetanic stimulation (3 × 100 Hz, 1 s) or theta bursts (4 × 100 Hz, 0.1 s) (data not shown), also failed to induced LTP. This absence of LTP resulted from the blockade of AMPARs since LTP could be induced before (51 ± 6%, $n = 12$) or after (+49 ± 8% $n = 12$) CNQX treatment (Fig. 1A). Likewise, LTP was also not facilitated by bicuculline methochloride (+12 ± 9%, $n = 8$) (Fig. 1B) but could be elicited by HFS after wash-out of CNQX (+67 ± 8%, $n = 7$). LTP was also not induced in presence of glycine (10 μM) that was applied to prevent a possible blockade of NMDARs by CNQX (+8 ± 4%, $n = 4$, with, and +3 ± 4%, $n = 5$ without bicuculline, respectively). Therefore, AMPARs are required for the induction of NMDAR-dependent LTP.

In contrast to these results, but in different experimental conditions, it was possible to induce LTP when AMPARs were transiently blocked. Thus, in agreement with Kauer et al. [9], LTP was readily induced (+49 ± 12%, $n = 8$) in

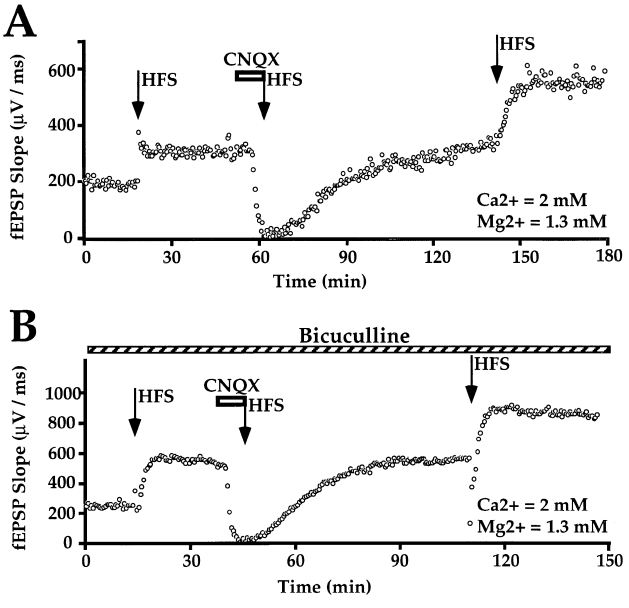

Fig. 1. Slopes of fEPSPs were recorded in ACSF (A) without, and (B) with bicuculline. Open bars correspond to the application of CNQX (10 μM, 6 min). Arrows indicate when the HFS has been delivered.

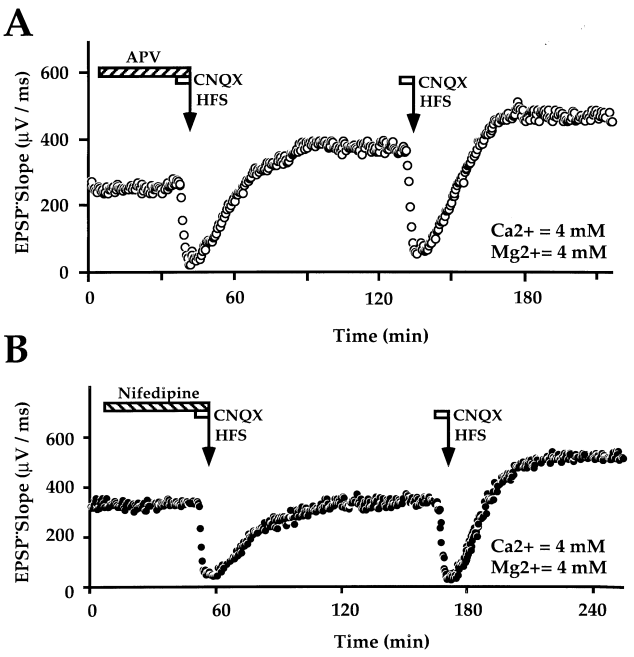

Fig. 2. Slices were superfused with ACSF4/4 containing 10 μM bicuculline. (A) DL-APV (100 μM) or (B) nifedipine (30 μM; hatched bars) were then superfused for 30 min alone, and for 6–10 min together with CNQX (10 μM, open bars). Note that nifedipine has no significant effect on fEPSP slope. A HFS (arrow) was applied when the fEPSP was completely suppressed. After wash-out of CNQX and stabilization of the fEPSP, a second HFS (arrow) was delivered in the presence of CNQX but without DL-APV (A) or nifedipine (B).

ACSF containing a high concentration of divalent cations (4 mM) in the presence of CNQX and bicuculline. To evaluate if this LTP is NMDAR-dependent, a high concentration of DL-APV (100 μM), was applied together with CNQX during the HFS and the two drugs were then washed-out. LTP was still observed (+38 ± 8%, $n = 6$). A second HFS applied without DL-APV but in the presence of CNQX, also generated a similar LTP (+51 ± 14%, $n = 6$) which was not significantly different ($P = 0.4$) from the previous one (Fig. 2A). Therefore, in the presence of 4 mM $CaCl_2$, 4 mM $MgCl_2$, LTP was essentially not NMDAR-dependent. As several data suggested that a voltage-dependent calcium channels (VDCC)-dependent form of LTP occurs in CA1 in non-physiological conditions, we evaluated this possibility in ACSF4/4. Application of HFS in the presence of CNQX plus nifedipine generated a modest LTP (+24 ± 6%, $n = 6$), significantly smaller ($P = 0.019$) than that observed when L-type VDCCs were not blocked (+49 ± 7%, $n = 6$) (Fig. 2B). Therefore, when AMPARs are transiently blocked, the LTP generated in high concentration of divalent cations and bicuculline is VDCC-dependent and not NMDAR-dependent.

What are the contributions of $GABA_A$ receptors to LTP induction? In keeping with earlier studies [5,14], a HFS first elicited a hyperpolarization followed by a large depolarizing response in CA1 pyramidal cells (+18 ± 3 mV, $n = 3$) in presence of AMPA and $GABA_B$ receptor antagonists. When

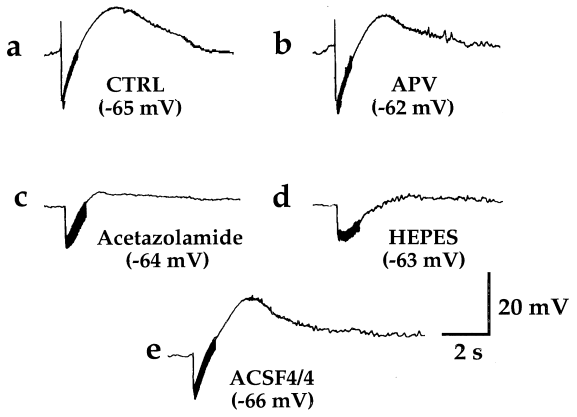

Fig. 3. Intracellular recordings were performed in CA1 pyramidal neurons at the holding potential indicated. Slices were first perfused with CNQX (10 μM) and CGP 35348 (1 mM). (a) Upon a HFS, a depolarization follows the hyperpolarized response. (b) Part of this response was reduced in the presence of DL-APV (100 μM). HFS was then applied to slices in the presence of AMPA, $GABA_B$ and NMDA receptor antagonists, in three different conditions: (c) preincubated for at least 30 min with acetazolamide (10 μM) in ACSF; (d) preincubated in HEPES gassed with pure oxygen for at least 1 h and superfused with the same buffer; (e) preincubated in ACSF4/4 for at least 1 h and superfused with the same buffer.

NMDARs were also blocked, the same sequence of events occurs with a depolarizing response of +14 ± 1 mV (n = 10) (Fig. 3). Both hyperpolarizing and depolarizing responses were blocked by bicuculline indicating that they are mediated by $GABA_A$Rs. The depolarization was specifically prevented by acetazolamide (10 μM), an inhibitor of carbonic anhydrase which blocks the enzyme-induced conversion of CO_2 into bicarbonate (+5 ± 1 mV, n = 5, $P < 0.0001$). A bicarbonate free buffer, such as HEPES buffer also blocked the depolarized response (+1 ± 1 mV, n = 7, $P < 0.0001$). Likewise, the specific GABAergic depolarizing response was also observed in ACSF4/4 (+16 ± 2 mV) but not in HEPES containing 4 mM of each divalent cation (+2 ± 1 mV, n = 3, P = 0.0022) (Fig. 3).

The NMDAR-dependent LTP induced in physiological ACSF without AMPA and GABA receptor antagonists (+47 ± 7%, n = 5) was not significantly different from that obtained in presence of acetazolamide (+45 ± 5%, n = 5) or in a HEPES buffer (+52 ± 7%, n = 6) (Table 1). The effects of acetazolamide and HEPES have not been examined when AMPARs were transiently blocked by CNQX during the tetanus since no LTP can be elicited in physiological conditions (see Fig. 1). In contrast, we have examined if the GABAergic depolarizing response could play a role in the VDCC-dependent LTP observed when AMPARs were blocked in non-physiological conditions (+52 ± 10%, n = 4). Again, LTP was prevented neither by acetazolamide (+52 ± 7%, n = 4), HEPES (+48 ± 12%, n = 4) nor bicuculline (+51 ± 14%, n = 6) (Table 1).

The lack of LTP observed in standard ACSF when AMPARs are blocked cannot be attributed to a possible blockade by CNQX of the glycine co-agonist site of NMDARs during the induction period since in the presence of exogenous glycine, which competitively protects NMDARs, LTP still could not be induced. These results are in agreement with those showing that the glycine site of the NMDAR is saturated by endogenous glycine [6]. The possibility that during HFS, a large release of glutamate overcomes the competitive blockade of AMPARS by CNQX has clearly been excluded previously [9]. Finally, artifacts due to a lack of plasticity of hippocampal slices or to a saturation of LTP are unlikely since LTP could be induced after the wash-out of CNQX in the same slice. The results suggest that in physiological ACSF, AMPARs are essential for the induction of NMDAR-dependent LTP, confirming the general belief.

The apparent contradiction raised by the work of Kauer et al. [9], resulted from the fact that they have used experimental conditions (high calcium and magnesium ions and a $GABA_A$R antagonist) leading generally to a NMDAR-dependent LTP when AMPARs were not blocked. They have implicitly assumed, but not demonstrated, that LTP was also NMDAR-dependent in the presence of CNQX. We now provided evidence that in these conditions LTP induction is not prevented by APV but is blocked by L-type VDCC antagonists. A NMDAR-independent form of LTP has also been reported in CA1 in ACSF4/4, even without CNQX [7] and several other reports have indicated that activation of VDCCs could contribute to the induction of NMDAR-independent LTPs in CA1 [1,4,7,8]. Nifedipine, which has no effect on NMDAR-dependent LTP [7], inhibited the induction of LTP when AMPARs were transiently blocked. Although the blockade of AMPARs also suppresses or decreases other sources of depolarization including NMDARs, activation of VDCCs is facilitated by high external concentrations of calcium ions and activation of metabotropic glutamate receptors [2,11,13]. Therefore, there is no contradiction between the general belief that AMPARs are required for the NMDAR-dependent LTP confirmed here, and the results of Kauer et al. [9] since two different forms of LTP are concerned.

The large and long-lasting GABAergic depolarization seen in CA1 pyramidal neurons following a HFS (Fig. 3)

Table 1
Contribution of $GABA_A$Rs to LTP induction in CA1

	LTP (%) ACSF, no CNQX	ACSF 4/4, CNQX
Control	47 ± 7 (n = 5)	52 ± 10 (n = 4)
Acetazolamide	45 ± 5 (n = 5)	52 ± 7 (n = 4)
Bicuculline	67 ± 8 (n = 8)	49 ± 12 (n = 8)
HEPES	52 ± 7 (n = 7)	48 ± 12 (n = 4)

LTP is not affected by blockade of the GABA-mediated depolarizing response. LTP was induced in ACSF or in ACSF4/4 and CNQX was added to transiently block AMPARs during HFS. Buffers containing acetazolamide were perfused at least 30 min before the HFS. Slices were first incubated for at least 1 h in HEPES and gassed with pure oxygen.

has been suggested to play a role in LTP induction under certain conditions [5,14,15]. The fact that bicuculline facilitates the induction of NMDAR-dependent LTP seems to play against this hypothesis but $GABA_AR$ antagonists blocked both the hyperpolarization but also the depolarization induced by HFS. In young rats where only the depolarizing response is present, the excitatory response mediated by $GABA_ARs$ lead to a postsynaptic depolarization which facilitates the activation of NMDARs [10]. Furthermore, in our conditions, the specific GABAergic depolarizing response was in the range of 14–20 mV and is probably sufficient to activate NMDARs. Nevertheless, reduction of this GABAergic depolarization by acetazolamide or its nearly complete block by HEPES ions, without modification of the hyperpolarization component, did not affect the induction of LTP (Table 1). This suggest that in normal ACSF, the depolarization provided by AMPARs is necessary and sufficient to induce NMDA-dependent LTP. The fact that no LTP could be induced in physiological conditions when AMPARs were blocked (Fig. 1) is in line with this suggestion. Furthermore, in non-physiological conditions (Table 1), the VDCC-dependent LTP which does not require AMPARs, was also not affected by acetazolamide and HEPES. Therefore, in adult rats, at least in the two conditions studied here, the depolarization provided by activation of $GABA_ARs$ during an intense neuronal activity appears not to be necessary for the induction of LTP in CA1.

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