TEA Elicits Two Distinct Potentiations of Synaptic Transmission in the CA1 Region of the Hippocampal Slice

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Extracellular application of tetraethylammonium (TEA) has been shown to elicit a prolonged synaptic potentiation in the CA1 region of the hippocampus that is unaffected by NMDA receptor antagonists, but is blocked by antagonists to voltage-dependent calcium channels (Aniksztejn and Ben-Ari, 1991; Huang and Malenka, 1993). In the present study the relation between TEA-induced potentiation and NMDA receptor-dependent long-term potentiation (LTP) was investigated in the CA1 region of the hippocampal slice using extracellular recordings and picrotoxin to block GABA_A-mediated inhibition. Consistent with the finding of Huang and Malenka (1993), NMDA receptor–dependent LTP partially occluded the TEA-induced potentiation. However, this occlusion was abolished when the NMDA receptor antagonist d(-)-2-amino-5-phosphonopentanoic acid (o-AP5) was present during the application of TEA, indicating one component of TEA-induced potentiation that is induced via NMDA receptor channels and another component that is distinct from NMDA receptor–dependent LTP. In the presence of antagonists to voltage-dependent calcium channels (nifedipine or nifedipine/flunarazine) application of TEA induced a potentiation that was largely occluded by NMDA receptor-dependent LTP. In common with NMDA receptor–dependent LTP, the TEA-induced potentiation, elicited in the presence of antagonists to voltage-dependent calcium channels, was associated with a symmetrical increase of the field EPSP. On the other hand, the TEA-induced potentiation elicited in the presence of o-AP5 produced an increase of the field EPSP that did not include the early part of the initial slope. It is concluded that application of TEA can activate both NMDA receptor channels and voltage-dependent calcium channels, and that the potentiation associated with activation of voltage-dependent calcium channels is distinct from NMDA receptor–dependent LTP.

[Key words: long-term potentiation, hippocampus, CA1, synaptic plasticity, tetraethylammonium, NMDA receptor, voltage-dependent calcium channel, calcium]

Long-term potentiation (LTP) of synaptic transmission is generally evoked by a brief afferent tetanus, this high-frequency activation providing the necessary coincident presynaptic activity to open postsynaptic NMDA receptor channels and allowing for calcium influx into the postsynaptic spine. The subsequent transient increase in calcium concentration locally in the postsynaptic spine is thought to be the trigger for LTP (Madison et al., 1991). Long-lasting potentiations of synaptic transmission can also be evoked by other means, for example by bath application of various drugs. An example of this is a transient application of the potassium channel blocker tetraethylammonium (TEA) (Aniksztejn and Ben-Ari, 1991). The TEA-induced potentiation is observed in the presence of antagonists to the NMDA receptor channel but is blocked by antagonists of voltage-dependent calcium channels (see also Grover and Teyler, 1991a). Alternatively, the TEA-induced synaptic potentiation may be distinct from the NMDA receptor–dependent LTP.

A recent study of TEA-induced potentiation reported a partial mutual occlusion between TEA-induced potentiation and the tetanus-induced one (Huang and Malenka, 1993). Whether the lack of full occlusion between the potentiations induced by tetanization and TEA, respectively, was due to unshared expression mechanisms or to the different induction conditions (bath application vs afferent tetanization), was left open by the authors. However, other studies using intense postsynaptic activation (Hess and Gustafsson, 1990), or application of NMDA (Asztely et al., 1991), have suggested the existence of mechanistically separate long-lasting synaptic potentiations in the CA1 region.

The aim of the present study was to examine whether the potentiation elicited by TEA is equivalent to the NMDA receptor–dependent LTP, or whether TEA elicits more than one potentiation process.

Materials and Methods

Experiments were performed on hippocampal slices prepared from 31 guinea pigs (300–400 gm) as previously described (Gustafsson et al., 1989). A surgical cut was made between CA3 and CA1 to prevent epileptiform bursting. The slices were maintained at 30°C, half submerged, in a constant flow (2–2.5 ml/min) chamber. The perfusion fluid contained (in mM) NaCl, 124; KCl, 4; CaCl_2, 2; MgCl_2, 2; NaHCO_3, 26; NaH_2PO_4, 1.25; glucose, 10; and gassed with 95% O_2, 5% CO_2. In experiments where GABA_A-mediated inhibition was blocked by 100 μM picrotoxin the extracellular concentrations of calcium and magnesium were raised to 4 mM each. Field potentials were recorded with glass micropipettes (filled with 3 M NaCl) in the apical dendritic layer of the CA1 region (stratum radiatum) and in the cell body layer. Two stimulation electrodes were positioned in the apical dendritic region on either side of the recording electrode to provide two independent afferent
inputs projecting to the same dendritic region. The two inputs were alternately stimulated at 0.5 Hz each. Test stimulation intensity was set to elicit a field EPSP initial slope that was about half that which just generated a population spike (measured in the cell body layer). Data were collected and analyzed on line (10 kHz sampling rate) using a 486 PC computer. The magnitude of the field EPSP was evaluated as initial slope, peak amplitude, and area (during 25 msec from EPSP onset), respectively. Area measurements gave essentially the same result as peak amplitude measurements and are therefore not presented. Field EPSP measurements were normalized with respect to the baseline and presented as the average of five consecutive measurements. The initial slope was calculated using linear regression of the first 0.6 msec just succeeding the presynaptic volley. LTP was induced by five 20 impulse trains at 50 Hz (intertrain interval, 10 sec) repeated twice with a few minutes interval. Stimulation intensity during these trains was set to the value at which a single stimulus just evoked a population spike. The values for LTP magnitude given in the Results were taken 15-25 min after the last tetanus. The values for the magnitude of the TEA-induced potentiation were taken 40-50 min after the onset of washout of TEA. Average values are given as mean ± SEM. Statistical significance for paired samples was evaluated using two-tailed Student's t test.

Picrotoxin, flunarazine, nifedipine, and tetraethylammonium chloride (TEA) were obtained from Sigma; D-2-amino-5-phosphono-pentanoic acid (D-AP5) was obtained from Tocris Neuramin. Drugs were applied to the perfusion line. Flunarazine and nifedipine were dissolved in ethanol (final concentration in the perfusion fluid 0.1%, v/v) and were protected from light.

Results
Effect of TEA application on the field EPSP
In agreement with previous studies (Aniksztejn and Ben-Ari, 1991; Huang and Malenka, 1993) a brief (6 min) application of 25 mM TEA was followed by a prolonged potentiation in the amplitude and initial slope of the field potential (Fig. 1A). The slow development of the potentiation after the washout of the drug was, at least partly, attributed to a transient (<30 min) reduction of the presynaptic volley and a delay in the onset of the field potential (Fig. 1A3,B3; see also Huang and Malenka, 1993). When measured after the end of these transient changes (40-50 min after the onset of TEA washout) the field potential peak amplitude was increased on average by 53% (Table 1); however, the increase in initial slope was only 15% (Table 1). This result indicates that TEA induced an asymmetric increase in the field potential, a given initial slope now associated with a 34% (144.9/115) greater amplitude. This discrepancy in initial slope and peak amplitude potentiation was observed also in the presence of picrotoxin (100 μM) in the perfusion fluid (Fig. 1B, Table 1), and was thus not related to alterations in GABAergic inhibition. Nevertheless, in the following the experiments.

| Table 1. TEA-induced potentiation in normal solution, in picrotoxin (100 μM), in picrotoxin + D-AP5 (50 μM), and in picrotoxin + nifedipine (20 μM)/flunarazine (30 μM) |
|----------------------------------|-----------------|-----------------|-----------------|
| Normal solution (n = 6)          | Initial slope   | Peak amplitude  | Amplitude/slope |
|                                  | 115.1 ± 3.4     | 152.7 ± 6.6     | 1.34 ± 0.09     |
| Picrotoxin (n = 9)               | 122.6 ± 4.1     | 144.9 ± 4.3     | 1.19 ± 0.03     |
| Picrotoxin + D-AP5 (n = 5)       | 102.4 ± 1.5     | 145.7 ± 7.7     | 1.47 ± 0.08     |
| Picrotoxin + nifedipine/flunarazine (n = 11) | 164.6 ± 6.0 | 160.0 ± 7.2 | 0.97 ± 0.02 |

Values are given as percentage of baseline ± SEM.
Figure 2. Interaction between tetanus-induced LTP and TEA-induced potentiation. A, Measurements of the peak amplitude (upper graph) and initial slope (lower graph) of the field EPSPs are shown from one experiment for a series of responses evoked at 0.5 Hz alternately to the test input (open circles) and to the control input (solid squares). Picrotoxin (100 μM) was present throughout the experiment. Three series of tetanizations (50 Hz, indicated by arrows) were delivered to the test input before the application of TEA (first three arrows), and to the control input after the application of TEA (last three arrows). The first tetanization to each input was a 10 impulse tetanus, the second and third sets of tetanizations to each input were five 20 impulse tetanis spaced at 10 sec. The test input stimulus intensity was reduced before the application of TEA so that the field EPSPs evoked by the test and control input were of approximately the same magnitude; 25 mM TEA was applied to the perfusion line for 6 min as indicated by the bar. In this experiment the field EPSP initial slope and amplitude of the test input were 109% and 133% of control, respectively, 40 min after the washout of TEA, and the corresponding values for the control input were 129% and 149%, respectively. B, Average (n = 20) records of the test and control field EPSPs taken at times indicated in A (a-e).

Table 2. Interaction between TEA-induced potentiation and tetanus-induced LTP in the presence of picrotoxin (100 μM)

|                  | Initial slope | Peak amplitude | Amplitude/slope |
|------------------|---------------|----------------|-----------------|
| Test input       | 101.6 ± 4.6   | 124.2 ± 7.6    | 1.22 ± 0.03     |
| Control input    | 122.6 ± 4.1   | 144.9 ± 4.3    | 1.19 ± 0.03     |
| Paired difference| 21.0 ± 4.4    | 20.7 ± 7.8     | -0.03 ± 0.05    |
| p                | <0.01         | <0.05          | >0.05           |

Values are given as percentage of baseline ± SEM. Before application of TEA the test input was tetanized, resulting in an average increase of the field EPSP initial slope to 196.6 ± 10.4%. The control input was tetanized after the establishment of the TEA-induced potentiation, which resulted in an increase to 192.4 ± 14.5%. p > 0.05, n = 9.
inputs became potentiated to much the same extent as the inputs tetanized prior to the TEA application (92.4% vs 96.6%, p > 0.05, n = 9).

**TEA-induced potentiation in the presence of an NMDA antagonist**

The above results suggest that TEA induces two distinct potentiations, one of which appears to be equal to tetanus-induced LTP. The question then arises whether this component of the TEA-induced potentiation is induced via activation of NMDA receptors, as is tetanus-induced LTP, or via some other route. To examine this question the same experiment as above was performed, with the exception that D-AP5 was added to the perfusion fluid in between the tetanization of the test input and the TEA application. Figure 3 (from one such experiment) and Table 3 show that under these conditions none of the inputs showed any significant TEA-induced potentiation in the initial slope. This result supports the notion that the TEA-induced potentiation in the initial slope is mediated via activation of NMDA receptors. Moreover, there was no significant difference in TEA-induced potentiation of the peak amplitude between the two inputs (Table 2), demonstrating that the asymmetric potentiation of the field EPSP induced by TEA is distinct from NMDA receptor–dependent tetanus-induced LTP.

**Table 3. Interaction between TEA-induced potentiation and tetanus-induced LTP in the presence of D-AP5**

|                | Initial slope | Peak amplitude | Amplitude/slope |
|----------------|---------------|----------------|-----------------|
| Test input     | 99.7 ± 1.4    | 140.8 ± 5.8    | 1.41 ± 0.07     |
| Control input  | 102.4 ± 1.5   | 145.2 ± 7.7    | 1.42 ± 0.08     |
| Paired difference| 2.7 ± 1.0    | 4.4 ± 3.1      | 0.01 ± 0.02     |
| p              | >0.05         | >0.05          | >0.05           |

Picrotoxin (100 µM) was present during the experiments. Values are given as percentage of baseline ± SEM. Before application of TEA the test input was tetanized, resulting in an average increase of the field EPSP initial slope to 210 ± 11.2% (n = 5).
involvement of voltage-dependent calcium channels in its induction. Under the present conditions TEA evoked a substantial prolonged potentiation also in the presence of either 20 μM nifedipine or the combination of 20 μM nifedipine and 30 μM flunarazine (Fig. 4, Table 4). Figure 4B and Table 4 show that this potentiation was associated with an about equal increase of field EPSP initial slope and amplitude, that is, a symmetrical increase in the field EPSP. As also shown in Table 4, the TEA-induced potentiation under these conditions was substantially reduced for inputs that had been tetanized prior to the TEA application.

**Discussion**

The present results suggest that the prolonged potentiation that follows a transient application of TEA is partially based on induction/expression mechanisms that are distinct from those underlying tetanus-induced LTP. The results support the notion that calcium ions through NMDA receptor channels have a privileged role in inducing NMDA receptor-dependent LTP. A corollary to this is that the prolonged potentiation induced by opening of voltage-dependent calcium channels is based on an expression mechanism distinct from NMDA receptor-dependent LTP.

The study of Aniksztejn and Ben-Ari (1991) showed that an LTP could be produced in a manner that bypasses the NMDA receptor channels. This notion was based on the finding that the generation of TEA-induced potentiation was unaffected by the presence of an NMDA receptor antagonist but was blocked by an antagonist to voltage-dependent calcium channels. Oclusion experiments to demonstrate whether this TEA-induced potentiation used the same expression mechanisms as NMDA receptor-dependent LTP was however not reported. More recently, oclusion experiments have indicated that TEA-induced potentiation and tetanus-induced LTP should share expression mechanisms, suggesting that NMDA receptor-dependent LTP indeed could be generated via calcium influx through voltage-dependent calcium channels (Huang and Malenka, 1993).

In general agreement with this latter study, the present one showed that TEA-induced potentiation was smaller in a previously tetanized input than in a naive one, indicating shared mechanisms. However, several findings suggest that this result might not necessarily be taken as evidence that NMDA receptor-dependent LTP can be evoked via calcium influx through voltage-dependent calcium channels. First, the result that TEA-induced potentiation was smaller in a previously tetanized input than in a naive one was not observed when TEA was applied in the presence of an NMDA receptor antagonist. This result implies that the partial occlusion between the TEA-induced potentiation and the tetanus-induced LTP (in the absence of an NMDA receptor antagonist) is related to the fact that TEA also induces potentiation via activation of NMDA receptor channels. Second, the finding of a similar magnitude of TEA-induced potentiation (in the presence of an NMDA receptor antagonist) in previously tetanized and naive inputs demonstrates that the TEA-induced potentiation uses an expression mechanism that is distinct from that underlying NMDA receptor-dependent LTP.

In agreement with the result that a component of the TEA-

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**Figure 4.** TEA-induced potentiation in the presence of antagonists to voltage-dependent calcium channels. A, Measurements of the peak amplitude (upper graph) and initial slope (lower graph) of the field EPSPs are shown from one experiment for a series of responses evoked at 0.5 Hz. Picrotoxin (100 μM) was present throughout the experiment; 25 mM TEA was applied to the perfusion line for 6 min as indicated by the bar. B, Average (n = 20) records of the test and control field EPSPs taken at times indicated in A (a and b).
induced potentiation was generated via NMDA receptor channel activation, a potentiation induced by TEA was also observed in the presence of antagonists to voltage-dependent calcium channels. This result contrasts with those of Aniksztejn and Ben-Ari (1991) and Huang and Malenka (1993), who found no significant potentiation in the presence of these antagonists. The TEA-induced potentiation presently observed in the presence of calcium channel antagonists was largely occluded by prior induction of tetanus-induced LTP, suggesting that it was indeed evoked via NMDA receptor activation. Moreover, this occlusion suggests, in agreement with Aniksztejn and Ben-Ari (1991) and Huang and Malenka (1993), that the TEA-induced potentiation (that does not interact with tetanus-induced LTP) is mediated via activation of voltage-dependent calcium channels. We have no explanation for the discrepancy regarding the ability of TEA to activate NMDA receptor channels except that the higher test stimulus rate and the presence of picrotoxin might have facilitated the activation of NMDA receptor channels (during the TEA application) in the present study (cf. Aniksztejn and Ben-Ari, 1991).

If, as suggested by the present results, the TEA-induced potentiation consists of two components, one might expect that the potentiation observed in the absence of any blocker would at least be the sum of that observed in the presence of antagonists to the NMDA receptor and the voltage-dependent calcium channels, respectively. However, this was not the case (compare Table 1). A tentative explanation for this behavior would be that the presence of one antagonist facilitates the induction conditions via the other route. In fact, calcium channel antagonists have been shown to cause increased synaptic activity in the hippocampal slice preparation (O'Regan et al., 1991). On the other hand, it less clear in what manner an NMDA receptor antagonist could facilitate activation of voltage-dependent calcium channels.

In support of the above notion, based on occlusion experiments and pharmacology, that TEA induces two separate poten- tiations using different expression mechanisms, the present analysis showed that TEA induces two different modifications of the field EPSP. One modification is associated with a symmetrical increase in the field EPSP (as is NMDA receptor-dependent LTP; Abraham et al., 1987; Asztely and Gustafsson, 1994), and the other one is asymmetric, the initial slope being much less affected than the remaining part of the field EPSP. This second component was not observed in the presence of calcium channel antagonists, and was the only one present in inputs that had been subjected to tetanization prior to the TEA application and when NMDA receptor antagonists was present. These results suggest that calcium influx through voltage-de-
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