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BRIEF COMMUNICATION

Pharmacology of Calcium-Induced Long-Term Potentiation in Rat Hippocampal Slices

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FRANK, C., S. SAGRATELLA, M. BENEDETTI AND A. SCOTTI DE CAROLIS. *Pharmacology of calcium-induced long-term potentiation in rat hippocampal slices*. PHARMACOL BIOCHEM BEHAV **33**(3) 713–715, 1989.—A transient increase (10 min) in extracellular calcium concentration (4 mM) causes a long-lasting (>2 hr) enhancement of population spike responses evoked by radiatum fibers to CA1 pyramidal neurons in rat hippocampal slices. This phenomenon is similar to tetanic long-term potentiation (LTP), and is also related to memory processes. The influence of various drugs was investigated on calcium-induced LTP. The NMDA antagonist 2 amino-5-phosphonopentanoic acid (AP5; 100 μM) was able to prevent the calcium-induced LTP, while atropine sulphate (10 μM), propranolol hydrochloride (10 μM) and verapamil hydrochloride (100 μM) were ineffective. The results suggest an involvement of the NMDA receptor in the development of calcium-induced LTP.

Calcium-induced LTP AP5 NMDA receptors

THE long-lasting increase in synaptic efficacy occurring after a brief tetanic electrical stimulus of presynaptic afferents is known as long-term potentiation (LTP). This phenomenon may be induced in a variety of central and peripheral tissues, especially in various regions of the hippocampus in experiments carried out in vivo and in vitro (1,12).

The long duration of LTP (10) and the other characteristics of this form of synaptic plasticity (14) suggest that LTP is related to memory processes (13). The mechanisms responsible for the induction of LTP are not yet well established. Nevertheless, it is clear that calcium ions play a role in its development. In fact, during the application of the electrical tetanus, low calcium (7) or "calcium-free" solutions (5) inhibit the induction of LTP. Furthermore, a short exposure of hippocampal slices to an elevated calcium concentration (4 mM) medium causes LTP that seems to be similar to tetanus-induced LTP (15).

The hippocampal calcium-induced LTP is suitable for pharmacological studies of the mechanisms underlying LTP, due to its time course characteristics and conditions of development (11).

In the present study we have studied the influence of various drugs affecting different neurotransmitter systems on the calcium-induced LTP in rat hippocampal slices, to elucidate the types of neurotransmitters involved in the development of this phenomenon.

METHOD

Male Wistar rats (250–300 g) were decapitated, the skull opened, and the hippocampus rapidly removed. Hippocampal slices of 450 μm thickness were cut with a tissue chopper, placed in a recording chamber, constantly perfused with an artificial cerebrospinal fluid (CSF) (122 mM of NaCl, 0.4 mM of KH_2PO_4, 3 mM of KCl, 1.2 mM of $MgCl_2$, 1.3 mM of $CaCl_2$, 25 mM $NaHCO_3$, 10 mM of glucose, pH 7.3); and then saturated with 95% O_2 and 5% CO_2. The temperature of the perfusion chamber was maintained at 33°C. The tested drugs were added to the perfused solution. Field potentials were recorded in the CA1 pyramidal cell layer, after stimulation of the Schaffer collaterals, at a rate of 0.1 Hz max.

Calcium-induced LTP was produced by raising the calcium concentration (4 mM) for 10 min. During this period the stimulation of Schaffer collaterals was interrupted. After restoring the

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TABLE 1

INFLUENCE OF DRUGS ON CALCIUM-INDUCED LTP

Drugs	N	t.10′ % Increase Control PS	t.30′ % Increase Control PS	t.60′ % Increase Control PS
Ca^{++} (4 mM)	8	115 ± 35	157 ± 49	185 ± 46
+				
AP5 (100 μM)	8	12.9 ± 4.3*	27.7 ± 9.5*	28.6 ± 11.2*
+				
Atropine (10 μM)	6	100 ± 30	165 ± 50	180 ± 48
+				
Propranolol (10 μM)	4	120 ± 29	170 ± 37	188 ± 25
+				
Verapamil (100 μM)	6	88.4 ± 20	163 ± 30	187 ± 35

*Significantly different from Ca^{++} alone $p<0.01$ according to ANOVA test and two-tailed Student's *t*-test; N = number of experiments; % increase control PS = % increase amplitude control population spikes; t.10′, t.30′, t.60′ = time at 10, 30 and 60 minutes from calcium perfusion.

calcium concentration to normal value, the modification of the amplitude of the CA1 population spike was observed for 60–90 min. 2-Amino-5-phosphonopentanoic acid (AP5; 50–100 μM), verapamil hydrochloride (50–100 μM), atropine sulphate (1–10 μM), and propranolol hydrochloride (1–5 μM) were included in the perfused solution during the induction of LTP to verify the influence of the drug on the calcium-induced LTP. In another set of experiments the effects of a 30 min perfusion of the drugs on the CA1 population spike in the absence of LTP were examined.

RESULTS AND DISCUSSION

The basal amplitude of CA1 population spike (ABFP) was not affected up to 30 min of drug perfusion by 50–100 μM of AP5 (ABFP 2.5 ± 0.6 mV before drug, 2.7 ± 0.5 mV after drug), 50–100 μM of verapamil hydrochloride (ABFP 1.2 ± 0.4 mV before drug, 1.5 ± 0.7 mV after drug), 1–5 μM of atropine sulphate (ABFP 2 ± 0.7 mV before drug, 1.8 ± 0.5 mV after drug) and 1–5 μM of propanolol hydrochloride (ABFP 2.6 ± 0.8 mV before drug, 2.8 ± 0.5 mV after drug).

The increase of the calcium concentration in the medium up to 4 mM resulted in a gradual increase of the amplitude of the CA1 population spike during all periods of observation (60–90 min), even after having restored the calcium concentration to 1.3 mM (Table 1).

Verapamil hydrochloride (50–100 μM), atropine sulphate (1–10 μM) and propranolol hydrochloride (1–10 μM) added for 10 min to the perfused solution containing high calcium levels (4 mM) did not affect the subsequent amplitude increase of the CA1 population spikes.

AP5 (50–100 μM) included in the perfused solution during the induction of LTP was able to significantly decrease the development of the LTP and, in 3 out of 9 slices tested, it was able to completely block the increase in amplitude of the CA1 population spikes (Table 1).

Many investigations have been carried out to further understand the LTP mechanism. To date it has been demonstrated that presynaptic (i.e., release of excitatory neurontransmitters) and postsynaptic mechanisms are involved in the generation of tetanus-induced LTP. The release of excitatory amino acid neurotransmitters during the depolarization of postsynaptic membranes remains the most widely accepted hypothesis for the development of CA1 tetanus-induced LTP (2,4). In fact, NMDA antagonists prevent the long-lasting increase of the synaptic efficacy after tetanic hippocampal electrical stimulation without influencing the normal synaptic activity (3,6).

In our study, the calcium-induced LTP (4 mM) was prevented by the NMDA antagonist AP5, while the muscarinic antagonist atropine and the beta adrenergic antagonist propranolol were ineffective.

These results confirm once more that in the calcium-induced potentiation of CA1 pyramidal cells evoked discharge, the NMDA receptors seem to play an important role in the development of the long lasting increase of CA1 field potentials.

Intracellular injection of the calcium chelator EGTA has been reported to block the tetanus-induced LTP of CA1 hippocampal cells (8). This finding suggests the important role of calcium ions in the induction of LTP. This prompted us to investigate the effect of verapamil on calcium-induced LTP.

Recent studies demonstrate that neurons have a number of different types of calcium channels, each of them with their unique pharmacological properties (9). In particular, while organic calcium antagonists such as nifedipine and verapamil were active on the L channel, inorganic calcium antagonists such as cadmium ions were able to block both N and L channels.

Verapamil did not prevent calcium-induced LTP, indicating a negligible role of the L calcium channel in the LTP in rat hippocampal slices. Hence, the N calcium channel involved in the release of neurotransmitters (9), and/or a receptor-operated calcium channel linked to NMDA receptors, might play a role in the genesis of calcium fluxes at the base of calcium-induced LTP.

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