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Changes of Free Fatty Acids and Acyl-CoAs in Rat Brain Hippocampal Slice with Tetraethylammonium-Induced Long-Term Potentiation

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We investigated the role of acyl-CoAs during induction and maintenance of long-term potentiation in rat brain hippocampus. Changes of acyl-CoA and free fatty acids (FFA) in hippocampus were measured during tetraethylammonium (TEA)-induced LTP. Results indicated that concentrations of acyl-CoAs and FFAs in slices were changed during TEA-induced LTP and 16:0-CoA and 18:0-CoA were increased in the early phase of stimulation, whereas free fatty acids in this phase were rather decreased. The increase of 20:4-CoA was delayed more than saturated acyl-CoAs. To examine the role of acyl-CoA in LTP of evoked transmitter release, we measured the glutamate release from hippocampal slice with the addition of acyl-CoA using glutamate electrode. Acyl-CoA (16:0-, 18:1-, and 20:4-CoA) could enhance glutamate release in hippocampal slice. It is suggested that saturated acyl-CoAs may play a functional role in the early phase of LTP. **© 2000 Academic Press**

Key Words: **long-term potentiation; tetraethylammonium chloride; free fatty acid; acyl-CoA; glutamate release.**

Tetraethylammonium chloride (TEA), a K^+ channel blocker, has been used to induce the long-term potentiation, a widely studied experimental model of learning (1). However, the precise mechanism of long-term potentiation in the hippocampus remained unresolved. There are evidences in favor of the three primary candidates: increased transmitter release, increased number of receptors and changes in spine or synapse morphology (2). An enhancement of glutamate release is a characteristic feature of long term potentiation (LTP) in hippocampus (3). Arachidonic acid (AA), nitric oxide (NO) and platelet activated factor (PAF) may play a role of retrograde messenger in the synaptic LTP (4–6). AA is released from the sn-2 position of membrane phospholipids by the activation of phospholipase A_2 (PLA_2) (7, 8).

Acyl-CoAs are important intermediates in lipid biosynthesis from fatty acids. Evidences have indicated that long-chain acyl-CoA ester has an important function in the regulation of intermediary metabolism and gene expression (9, 10).

Until now, however, it was not reported whether acyl-CoA played a significant role in the LTP of hippocampus. In the present study, we measured the change of acyl-CoAs and free fatty acids in hippocampal slice following induction of LTP. To examine the role of acyl-CoA in LTP of evoked transmitter release, we have investigated the release of glutamate from slice following addition of acyl-CoA by monitoring of real-time glutamate release using glutamate electrode. Our results indicated that the change of amount of acyl-CoAs and free fatty acids in slices took place during TEA-induced LTP in various fashions depending on acyl-chain characteristics. Acyl-CoAs (16:0-, 18:1-, and 20:4-CoA) could enhance glutamate release from hippocampal slice. We conclude that acyl-CoAs may play a role of neural regulator in the early phase of LTP.

MATERIALS AND METHODS

Preparation of hippocampal slices. Hippocampal slices were prepared from female Wistar rats (6 weeks old). The procedure reported by Hosokawa *et al.* (11), with some modifications, was used to prepare hippocampal slices. Rats were decapitated, and brains were rapidly removed and then submerged briefly in ice-cold oxygenated calcium-free artificial cerebrospinal fluid (ACSF) containing 120 mM NaCl, 3 mM KCl, 1.2 mM NaH_2PO_4, 23 mM $NaHCO_3$, 2.4 mM $MgCl_2$, and 11 mM D-glucose, pH 7.3. The hippocampus tissue was then dissected out and prepared to obtain transverse hippocampal slices (400 μm thickness), using a hand-made chopper using two nibs. The slices were incubated in continuously oxygenated standard ACSF, at room temperature for 2 h.

Abbreviations used: LTP, long-term potentiation; FFAs, free fatty acids; TEA, tetraethylammonium chloride; *x*:*y*-CoA, fatty acyl-CoA with carbon number *x* and the extent of unsaturation *y*.

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Electrophysiological record of TEA-induced LTP. Electrophysiological record was carried out using a method reported by Liu *et al.* (12). In brief, after 2 h incubation in standard ACSF, a slice was transferred to an interface recording chamber and perfused at a flow rate of 2 ml/min with standard ACSF that was warmed at 32°C. After obtaining of a stable baseline of field excitatory postsynaptic potentials (e.p.s.ps), long-term potentiation was produced by a change of the superfusate from standard to 25 mM potentiation medium (124 mM NaCl, 5 mM KCl, 1.25 mM KH_2PO_4, 24 mM $NaHCO_3$, 0.1 mM $MgCl_2$, 5 mM $CaCl_2$, 10 mM D-glucose, and 25 mM tetraethylammonium chloride, pH 7.2) for 10 min. After potentiation the standard ACSF superfusion was continued for the remainder of the experiment.

Acyl-CoA analysis. A method reported by Deutsch *et al.* (13), with some modifications, was used to assay acyl-CoA in hippocampal slices. Slices were incubated for 2 h in standard ACSF. The temperature of medium was changed from the room temperature (~25°C) to 32°C and maintained for 30 min. The medium was changed to potentiation medium (25 mM TEA) to induce LTP and maintained for 2, 5, 10, 15, and 20 min. As a control, the slice without TEA was maintained for the same period at the same temperature as the potentiated slice. Then the medium containing slices was rapidly transferred to ice bath and reduced pH of the potentiation medium to 4.5 to terminate reaction of acyl-CoA synthesis. The slices were immediately homogenized by a Teflon–glass homogenizer in 25 mM KH_2PO_4 (pH 4.5, 2 ml) kept on ice. The protein concentration of the homogenate was measured by using BCA protein assay reagent (Pierce, U.S.A.). The internal standard, 17:0-CoA (4.4 nmol), was added and the mixture was sonicated for 20 s in bath-type sonicator (100 W). Next 2 ml of isopropanol was added, and then the sample was sonicated again for 20 s. Saturated $(NH_4)_2SO_4$ (0.25 ml) and acetonitrile (4 ml) were added and vortexed for 2 min and centrifuged for 5 min (3000 rpm). 25 mM KH_2PO_4 (10 ml) was added to the supernatant. Acyl-CoAs were extracted and purified using Oligonucleotide Purification Cartridge (Applied Biosystem Division, Foster City, CA) and assayed by HPLC system comprised of tunable absorbance detector (Tosoh, Japan; UV-8010) set at 260 nm. Separation was performed on a column (TSK-GEL ODS-120T, 4.6 × 250 mm, Tosoh, Japan). Chromatography was performed with a gradient system including two mobile phases: (A) 75 mM KH_2PO_4, pH 4.5, and (B) 100% acetonitrile. The starting conditions were 1.0 ml/min flow rate and 44% B phase. B phase then was increased to 49% over 5 min, and was kept at 49% for 20 min, then increased to 70% over 25 min and was kept at 70% for 5 min.

Recording of glutamate release. After 2 h incubation in standard ACSF, a slice was transferred to a culture insert with fine nylon mesh soaked in a magnetic-stirred standard ACSF saturated with 95% O_2 plus 5% CO_2. The glutamate electrode (Sycopel International Ltd. England) filled with L-glutamate oxidase was placed on CA1 region of the rat hippocampal slice to measure the real-time release of glutamate. The glutamate electrode was attached to the electrochemical detector (EICOM EPS-800, Japan). Then, glutamate release was detected by the addition of 1 μL of 1.6 mM acyl-CoA solutions with the aid of a syringe injector.

Analysis of free fatty acid in hippocampal slices. The procedure reported by Yoshida *et al.* (14), with some modifications, was used to analyze free fatty acid. Slices exposed in 25 mM TEA potentiation medium for 2 min, 5 min, 10 min, 15 min, and 20 min were homogenized in 2 ml of 25 mM KH_2PO_4 (pH 4.5) kept on ice. Protein content of homogenate was measured. The internal standard of heptadecanoic acid (4.4 nmol) and hexane (10 ml) was added, and the mixture was vigorously shaken for 1 min and sonicated for 30 s. The mixture was then centrifuged for 5 min (3000 rpm) and fatty acids extracted in hexane phase were analyzed by the on-column methylation method (now we used Meth Prep (GL Science Inc. Japan)) developed by MacGee and Allen (15) using the gas chromatography-mass spectrometry (QP5000; Shimadzu, Kyoto, Japan).

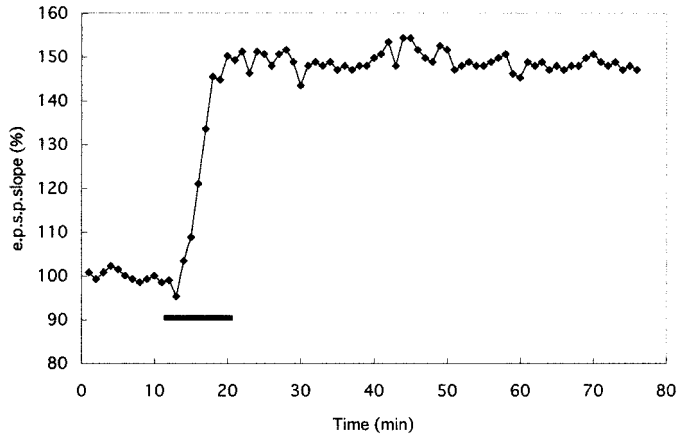

FIG. 1. The graph shows the record of slope of evoked field excitatory postsynaptic potentials from a rat hippocampal slice by the addition of 25 mM TEA potentiation medium for 10 min. The potentiation resulted in an immediate and sustained increase in the slope of field excitatory postsynaptic potentials.

RESULTS

After changing of medium from standard ACSF to 25 mM TEA potentiation medium, the synaptic response from slice was shown in Fig. 1, confirming the result presented by Hosokawa *et al.* (11). After addition of 25 mM TEA potentiation medium within 10 min, a persistent enhancement of the synaptic potentiation was observed.

The ratios of each acyl-CoA concentrations after stimulated by 25 mM TEA with duration for 2, 5, 10, 15 and 20 min to the corresponding control (without TEA and with the corresponding duration) were shown in Fig. 2a. These results indicate that the concentration of stearoyl-CoA (18:0-CoA) in potentiated slices was obviously increased as compared to control slices after 2 min stimulation of TEA, whereas there was no significant difference of 18:0-CoA concentration between control and potentiated slices 5, 15, 20 min after stimulation of TEA. After 10 min stimulation by TEA, 18:0-CoA concentration in stimulated slices was decreased as compared to control slices. An obvious increase of arachidonoyl-CoA (20:4-CoA) concentration occurred after 10 min stimulation of 25 mM TEA, while there was no significant difference of 20:4-CoA concentration between control and potentiated slices 2, 5, 15 and 20 min after stimulation of 25 mM TEA. There was no significant change in palmitoyl-CoA (16:0-CoA) and oleoyl-CoA (18:1-CoA) concentration between control slices and stimulated slices by 25 mM TEA with duration for 2, 5, 10, 15, and 20 min; however, the tendency of 16:0-CoA change was similar to that of 18:0-CoA change.

Relative changes of free fatty acids in slices were shown in Fig. 2b, which shows the time dependent changes of free fatty acids presented in the ratio be-

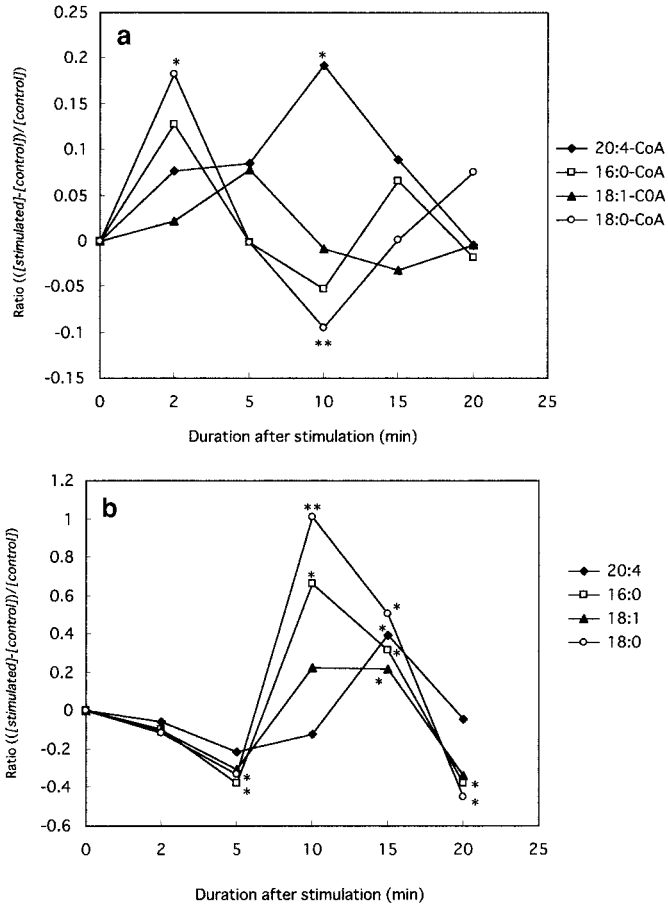

FIG. 2. (a) Relative changes, presented as the ratio between the concentration of acyl-CoA in the slice after stimulation by 25 mM TEA ([stimulated]), and that in the control slice ([control]) were shown. Each of acyl-CoA in stimulated slices was compared with the corresponding control slice and the ratio was calculated (([stimulated] − [control])/[control]). Significant differences are indicated in * ($P < 0.05$) and ** ($P < 0.01$) for 4 experiments. The change of 20:4-CoA at 10 min was significant ($P < 0.05$) and the change of 18:0-CoA were also significant at 2 min ($P < 0.05$) and 10 min ($P < 0.01$). (b) Relative changes in free fatty acids. Each free fatty acid in the stimulated slice was compared with the corresponding control. Significant differences are indicated in * ($P < 0.05$) and ** ($P < 0.01$) for 4 experiments.

tween the TEA-stimulated group and the corresponding control group for a given fatty acid. These results showed that there was no significant change or rather decrease in each concentration of palmitic, stearic, oleic and arachidonic acids in stimulated slices as compared to the control after 2 min of stimulation by addition of 25 mM TEA. After stimulation for 15 min by TEA, all of palmitate, stearate, oleate and arachidonate in potentiated slices were significantly increased as compared with the control. Concentration of palmitate and stearate in stimulated slices was significantly decreased within 5 min and 20 min after stimulation as compared with control slices, while there was no change of the concentration of 18:1 and 20:4 as compared with the control slices. After 10 min of stimulation by TEA, the concentration of 16:0 and 18:0 in stimulation group was more than that of control group, however there was no difference in concentration of 18:1 and 20:4 between the control and stimulated slices.

The pattern of glutamate release in the rat hippocampal slice induced by addition of acyl-CoA was presented in Fig. 3, which showed that real-time glutamate release was recorded when 18:1-CoA and 20:4-CoA were alternatively added to slice.

DISCUSSION

It was reported that arachidonic acid played a role of a retrograde messenger during induction of LTP (4). However, there was not evidence whether the change of acyl-CoA concentration in hippocampus occurred during induction of LTP. Acyl-CoAs may be rapidly formed from free fatty acids by acyl-CoA synthetase as an intermediate for the synthesis of phospholipids. Acyl-CoA concentration was determined in slices from the control and chemically potentiated hippocampal slices induced by 25 mM TEA with duration of 2, 5, 10, 15 and 20 min. There was a significant increase in concentration of 18:0-CoA and 16:0-CoA in nearly the same extent in hippocampal slices in earlier phase of

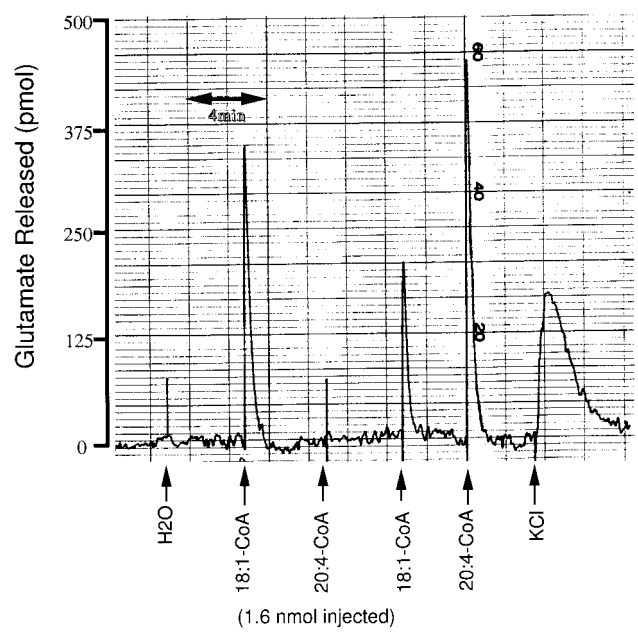

FIG. 3. The glutamate–electrode response patterns of glutamate release from rat hippocampal slice by the addition of acyl-CoA. Here a typical experiment was presented, where 18:1-CoA and 20:4-CoA were added with two repeated injections and 2 μL of 1 M KCl was added for observation of glutamate release by KCl-dependent depolarization of hippocampus. This addition of KCl resulted in evacuation of glutamate from the slice under the present experimental condition. We will report quantitative results in elsewhere.

stimulation by TEA than 20:4-CoA. Our results indicated that 20:4-CoA in stimulated slices was increased only after 10 min of stimulation, whereas 18:0-CoA concentration was decreased significantly at that time. There was no significant change of each acyl-CoA concentration between the control and the stimulation groups after 15–20 min of stimulation. These results may indicate that acyl-CoA synthetase is present in two independent free fatty acid pools, which would contain saturated (or monounsaturated) fatty acids in one pool and polyunsaturated fatty acids in the other. An increase of the concentration of 18:0-CoA in slices after 2 min stimulation of TEA with relatively decreasing the amount of free stearic acid may be explained by the early phase activation of acyl-CoA synthetase in the pool of saturated fatty acids in the early phase. On the other hand, at 10 min after stimulation of TEA, the activation of acyl-CoA synthetase in the pool of polyunsaturated fatty acids would result the increase of 20:4-CoA concentration in the potentiated slices, whereas the decrease of 18:0-CoA in the stimulated slice at 10 min would be explained by the decrease of acyl-CoA synthetase activity in the pool of saturated fatty acids and 18:0-CoA would be further metabolized to form phospholipids.

The assumption that phospholipase A_1 and phospholipase A_2 activities changed differently in the time course would explain changes of free fatty acids in potentiated slices. At 10 min after stimulation of TEA, the concentration of palmitate and stearate in potentiated slices were increased significantly possibly by the activation of phospholipase A_1, which was activated in more earlier phase of time course than the activation of phospholipase A_2. At 15 min after stimulation of TEA, the release of arachidonic acid was maximized, and this may be explained by the activation of phospholipase A_2. It is clear that the release of saturated acyl-CoAs occurred in LTP prior to the release of free fatty acids including arachidonic acid.

Rabin *et al.* (16) and Deutsch *et al.* (17) reported changes of brain acyl-CoA and free fatty acid concentrations following ischemia–reperfusion. In their papers, only arachidonoyl-CoA concentration was increased at 3 min after decapitation (17) and 20:4- and 18:0-CoA were increased in the ischemia–reperfusion animals (16) with unaffected total acyl-CoA concentration, whereas free fatty acid concentrations were increased. These results show that reacylation–deacylation cycles in the rat brain may work differently for neural stimulation by decapitation and ischemia–reperfusion system.

The presence of pool of 16:0-CoA owing to release and recycling of unlabeled fatty acids from phospholipid breakdown was reported by Grange *et al.* (18). The sources other than the plasma unesterified fatty acid pool may contribute to brain fatty acid incorporation and the turnover rates of palmitate in brain phospholipids are much greater (18). In ischemia–reperfusion brain, the formation of phospholipids from free fatty acid pool is not a steady state (16). Our results would also suggest that free fatty acid and acyl-CoA pools are dynamically changed during TEA-induced LTP. Clements *et al.* (19) reported that there was a significant increase in the concentration of free arachidonic acid in dentate gyrus following tetanic-LTP. Although Hanse *et al.* (20) reported that TEA-induced LTP and tetanic-LTP share similar mechanisms, we should further clarify whether an increase in the acyl-CoA production may also occur or not in the tetanic LTP with a special microassay technique for acyl-CoAs, which may be difficult at present.

Recently it was reported (21) that the incorporation of arachidonoyl-CoA to produce phosphatidic acid was necessary to enhance endocytosis, whereas palmitoyl-CoA acted suppressive. We also detected glutamate release from the hippocampal slice by addition of acyl-CoAs (16:0-CoA, 18:1-CoA and 20:4-CoA) (Fig. 3) but not by free arachidonic acid (not shown in this paper), which indicates that the increase of acyl-CoA may stimulate the neurotransmitter release in hippocampal slice. We can not rule out the role of glial cells in regulation of glutamate release. Actually arachidonic acid plays a role to inhibit glutamate uptake into glial cells (22). Further works may be needed to elucidate the role of glia on glutamate release and regulation by fatty acids and acyl-CoAs.

In summary, we have evaluated changes in free fatty acid and acyl-CoA concentrations in rat hippocampal slices with TEA-induced LTP. Addition of acyl-CoA enhanced glutamate release from hippocampal slice. These results suggest that acyl-CoA may play a role of neural regulator in LTP of hippocampus.

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