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FATTY ACIDS MODULATE EXCITABILITY IN GUINEA-PIG HIPPOCAMPAL SLICES

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Abstract—A variety of fatty acids produced sustained changes in excitability in the guinea-pig hippocampal slice. Although each fatty acid was unique, a general pattern was evident. During a 30-min exposure, the synaptic potential was minimally affected, although population spike amplitude showed significant increases. With wash, synaptic efficacy increased. The increase in the synaptic potential was significant with arachidonic acid (100 μ M), oleic acid (100 μ M), myristic acid (250 μ M) and capric acid (250 μ M). Also with wash, the coupling between the synaptic potential and the population spike was reduced significantly for most of the fatty acids tested: arachidonic acid (50 μ M, 100 μ M), linoleic acid (100 μ M), oleic acid (100 μ M), stearic acid (100 μ M), myristic acid (250 μ M) and capric acid (250 μ M, 500 μ M).

The fatty acids may influence neuronal excitability, in part, through a direct membrane action. The observed synaptic enhancement is consistent with a role for a fatty acid in long-term potentiation. In addition, fatty acid exposure mimics the effects of X-radiation. We suggest that free radical-induced release of fatty acids contributes to electrophysiological damage in a number of pathological states.

Exposure to free radicals, and consequent lipid peroxidation, causes the activation of phospholipases and the release of fatty acids from cellular membranes.^{7,8,27,34} Oxidized membrane lipids are preferentially, but not exclusively, removed by phospholipase A2 and repaired by glutathione peroxidase.³⁴ In rat cortical brain slices, arachidonic acid, oleic acid and docosahexaenoic acid were released following superoxide-induced lipid peroxidation⁸ and phospholipase A2 activation.⁷ These mechanisms can come into play during some pathological conditions. For example, ischemic injury causes the release of free fatty acids,^{1,4,39} probably as a consequence of free radical generation.^{29,39} The release of arachidonic acid is greater in field CA1 than in field CA3 of hippocampus corresponding to the severity of ischemic injury.³⁵

Recent reports have shown that fatty acids have a wide variety of effects on membrane currents in excitable tissue. Among these actions are increased potassium currents^{5,12,13,20} and decreased sodium current.^{15,30,31} Arachidonic acid and its lipoxygenase metabolites have been shown to modulate synaptic excitability.^{6,11,28,38} A role for arachidonic acid or oleic acid as an essential second messenger in long-term potentiation (LTP) has been postulated.^{11,14,16,37,38}

Free radical exposure has been shown to have electrophysiological consequences in neural tissue.^{21,23,26,33} The present experiments were initiated to evaluate the role of fatty acids in free radical damage in the guinea-pig hippocampal slice. Among the fatty

acids we tested were arachidonic acid (20:4), linoleic acid (18:2) and oleic acid (18:1) which are released by free radical exposure.⁸ In addition, we chose a series of saturated fatty acids of increasing length (10:0, 14:0, 18:0) and a series of 18-carbon fatty acids with increasing unsaturation (18:0, 18:1, 18:2). Preliminary results have been reported previously in abstract form.²³

EXPERIMENTAL PROCEDURES

Slices of hippocampus were prepared from brains of male Hartley guinea-pigs (Harlan Sprague Dawley, Inc.) as described previously.^{21,26} Slices were incubated at room temperature for at least 1 h, placed in a submersion slice chamber and perfused at approximately 1 ml/min with oxygenated artificial cerebrospinal fluid (ACSF) at $30 \pm 1^\circ\text{C}$. ACSF had the following composition (in mM): NaCl, 124; KCl, 3.0; CaCl_2 , 2.4; MgSO_4 , 1.3; KH_2PO_4 , 1.24; glucose, 10; and NaHCO_3 , 25; equilibrated with 95% O_2 -5% CO_2 .

Fatty acids were put into solution immediately prior to perfusion through the bath. Arachidonic acid, oleic acid and linoleic acid were received from the supplier (NuChek or Sigma) in sealed vials. After opening the vial, the full content was dissolved in ethanol (99%), separated into aliquots, and stored under nitrogen at -70°C for not more than one week. Immediately before use, the stock solution was removed from the freezer and diluted in ACSF to a final concentration of 0.3% ethanol and 50–250 μ M fatty acid. Stocks were never refrozen following thawing for use. Myristic acid did not stay in solution when diluted from an ethanol stock; dimethylsulfoxide (DMSO; 0.3% final concentration) was used instead. Stearic acid was minimally soluble with either solvent; solutions were used with the fatty acid in suspension with ethanol. Capric acid was used as the sodium salt and directly dissolved in the oxygenated ACSF.

The actions of ethanol (0.3%, $n = 9$), DMSO (0.3%, $n = 6$) and time alone ($n = 11$) were evaluated on electrophysiological responses in hippocampal slices. With time

Abbreviations: ACSF, artificial cerebrospinal fluid; DMSO, dimethylsulfoxide; E/S, excitatory postsynaptic potential-spike; I/O, input-output; LTP, long-term potentiation; PSP, postsynaptic potential.

and solvents the synaptic response was slightly reduced and excitatory postsynaptic potential-spike (E/S) coupling (see below) was enhanced. Although these actions were small, they were statistically significant. Consequently, all data were referenced to the appropriate solvent control.

Perfusion with the fatty acids had to be watched carefully to avoid reduced flow and consequently decreased bath temperature. In this study all slices were constantly monitored with a Yellow Springs Instruments temperature probe and the perfusion rate was checked at frequent intervals. Any slice in which large changes occurred was deleted from the study. At least five experiments were done with each fatty acid at each dose: arachidonic acid, 50 μ M: $n = 6$; 100 μ M: $n = 7$; linoleic acid, 100 μ M: $n = 5$; oleic acid, 100 μ M: $n = 5$; stearic acid, 100 μ M: $n = 8$; myristic acid, 100 μ M: $n = 5$; 250 μ M: $n = 6$; capric acid, 100 μ M: $n = 5$; 250 μ M: $n = 6$.

A bipolar stainless-steel stimulating electrode was positioned in the stratum radiatum of field CA1 to stimulate afferent pathways. Recording electrodes (2 M NaCl) were placed in the stratum radiatum and in the stratum pyramidale to record the resultant population postsynaptic potential (PSP) and the population spike, respectively. The field potentials were recorded with high gain d.c. amplifiers (WPI) and were digitized, stored and analysed on a PDP 11-73 microcomputer. Population PSPs were quantitated by their initial slope to avoid complications caused by the reflected population spike in stratum radiatum.

Input output (I/O) curves were generated by constant current stimulation (0–0.5 mA, 300 μ s) of stratum radiatum in field CA1. As previously described,³³ average I/O curves were constructed for each experimental condition by computing the mean and standard errors for the population spike amplitude, synaptic potential slope and afferent volley amplitude at each stimulus intensity. Three relationships were analysed. (1) Plotting afferent volley vs the initial slope of the population PSP reflects the ability of presynaptic fibers to elicit a synaptic response. This relationship will be referred to as synaptic efficacy. (2) The relationship between the population PSP slope and population spike amplitude has been called E/S coupling³ and reflects the ability of the synaptic potential to evoke a population spike (i.e. spike generation). Changes in E/S coupling are functionally distinct from changes in synaptic efficacy.^{2,9,32} A variety of mechanisms has been proposed,^{2,9,32} including altered postsynaptic membrane potential, modified postsynaptic membrane excitability and altered inhibitory synaptic input. In the present study, changes in this set of curves are descriptive of an effect but do not attempt to describe a mechanism. (3) The relationship of afferent volley vs population spike amplitude encompasses both changes in synaptic efficacy and E/S coupling. An increase in population spike amplitude at a fixed afferent volley size could result from either a larger synaptic response or an increase in E/S coupling.

I/O curves were obtained in normal ACSF after a 30-min equilibration period. Slices were then exposed to the fatty acid solution for 30 min and another I/O curve obtained. The tissue was subsequently washed with normal ACSF for a minimum of 30 min. I/O were obtained approximately every 30 min during this wash period. I/O curves were analysed as previously described.³³ Each curve was computer-fit with the equation for a sigmoid curve and differences between curves were quantified by the comparison of the ratio (a/c) of two parameters defining the best-fit curves (RS 1, BBN Software Product, Cambridge, MA). a is the maximal y value and c is the x value at half-maximal y . Under the experimental conditions in previous studies^{24–26,33} a decrease in a was usually accompanied by an increase in c , representing a net decrease in the relationship. In the present study the relationship between population PSP and population spike showed increases in both parameters. In this case, the parameters were also evaluated separately. Data were compared by Student's t -test to the appropriate

solvent controls and presented as a percentage of these controls. Standard errors were computed from the errors associated with the parameters of both the experimental and the control fitted curves. Significance was accepted at $P < 0.05$.

RESULTS

Enhancement of synaptic response

Exposure and subsequent washout of a variety of fatty acids was found to produce a sustained increase in the synaptic potential in field CA1 of guinea-pig hippocampus. Capric acid (250 μ M) caused the largest enhancement (37% compared to the ACSF time control, Fig. 1B). Arachidonic acid (100 μ M) (Fig. 1A), myristic acid (250 μ M) and oleic acid (100 μ M) also significantly increased the ability of a given volley size to elicit a synaptic potential, while stearic acid (100 μ M) and capric acid (100 μ M) were ineffective. Linoleic acid (100 μ M) and myristic acid (100 μ M), respectively, averaged a 19.4 and 24.3% increase in synaptic efficacy beyond the solvent

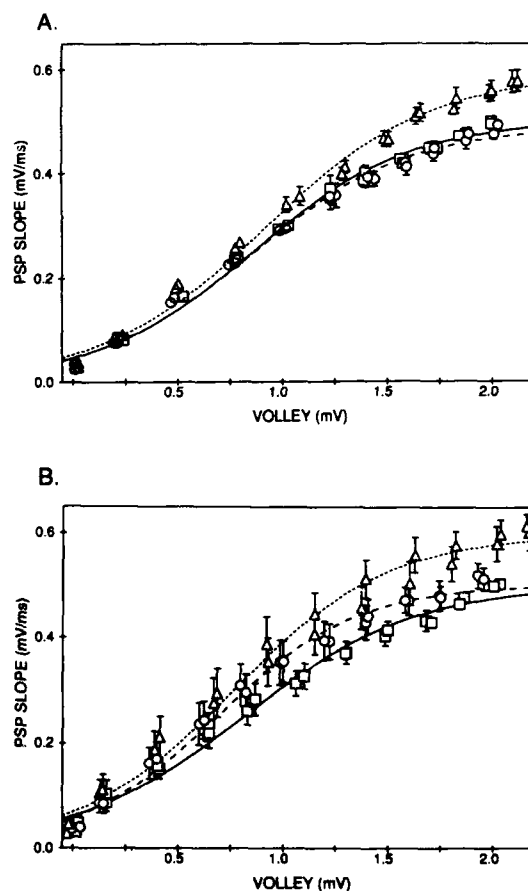


Fig. 1 Fatty acids enhance synaptic efficacy. Plot of afferent volley vs population PSP size reflects synaptic efficacy of the afferent pathway to the CA1 pyramidal cells. (A) Average curve from seven hippocampal slices treated with 100 μ M arachidonic acid. $\square$, single line: control; $\circ$, dashed line: during 30-min application of fatty acid; $\triangle$, dotted line: 30-min wash with normal solution. (B) Average curve for six slices treated with 250 μ M capric acid.

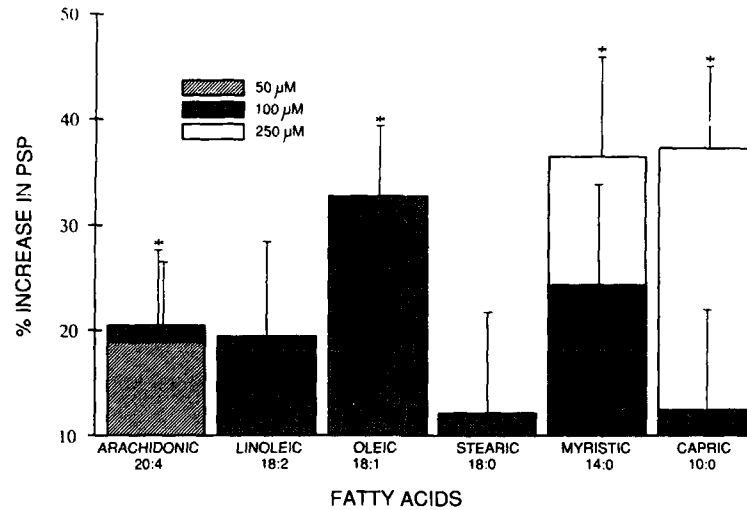


Fig. 2. Many fatty acids have similar effects on increasing the population PSP with wash. The change was significant following exposure to arachidonic acid (100 μ M), oleic acid (100 μ M), myristic acid (250 μ M) and capric acid (250 μ M). All changes are calculated in reference to the appropriate solvent or time control.

controls, but neither achieved statistical significance (Fig. 2).

During the exposure to the fatty acids, there was no significant change in the population PSPs (Fig. 1), yet all but 50 μ M arachidonic acid seemed to produce a slight increase. Oleic acid, myristic acid and capric acid showed increases between 10 and 25% relative to the appropriate solvent controls but were not statistically significant. With 50 μ M arachidonic acid, a depression predominated, seen as a small reduction in the ability of the afferent volley to evoke a population PSP. Again, these small changes were not statistically significant. Our results are consistent with those of Drapeau *et al.*¹¹ who showed that arachidonic acid could depress the synaptic response during the exposure to the fatty acid, although in their experiments potentiation predominated.

Altered excitatory postsynaptic potential-spike coupling

In addition to the enhancement of the synaptic response with washout of the fatty acids, a significant shift in E/S coupling occurred. The relationship between the synaptic potential and the population spike was significantly shifted to the right for all the fatty acids tested except 100 μ M myristic acid and 100 μ M capric acid (Fig. 4). The increase ranged from 11% with 100 μ M linoleic acid to 33% with 50 μ M arachidonic acid compared to the appropriate solvent controls. As with the synaptic potentiation, the shift in E/S coupling was greatest following washout of the fatty acid. During the exposure period, only 250 μ M myristic acid produced a significant shift in this curve (Fig. 3B). The change in the E/S relationship with 50 μ M arachidonic acid and 250 μ M myristic acid is shown in Fig. 3. The shift in the curves (triangles, dotted line) to the right are small but significant compared to the paired

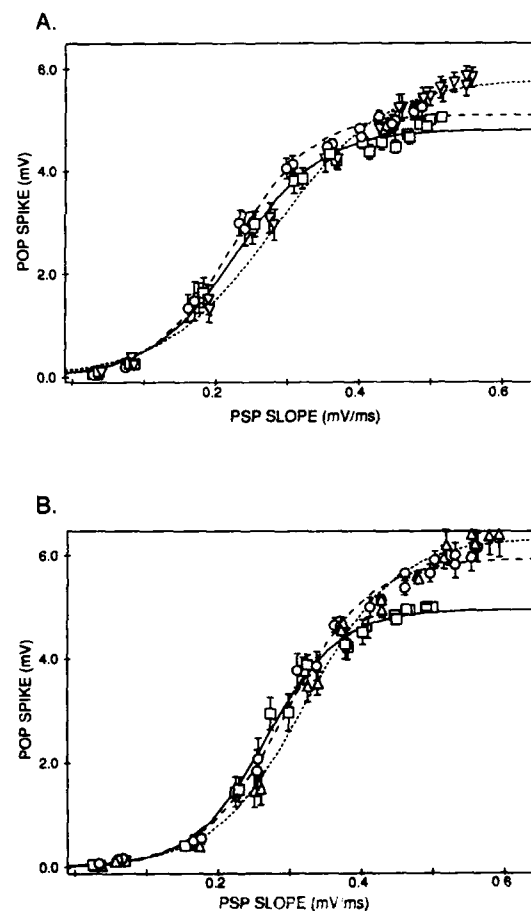


Fig. 3. Plots of population PSP size vs population spike amplitude show that fatty acids alter the ability of a synaptic potential to generate a spike. (A) Average curve ($n = 6$) showing effect of exposure and washout of 50 μ M arachidonic acid. $\square$, single line: control; $\circ$, dashed line: during 30-min application of fatty acid; $\triangle$, dotted line: 30-min wash with normal solution. (B) Average curve ($n = 6$) for slices exposed to 250 μ M myristic acid.

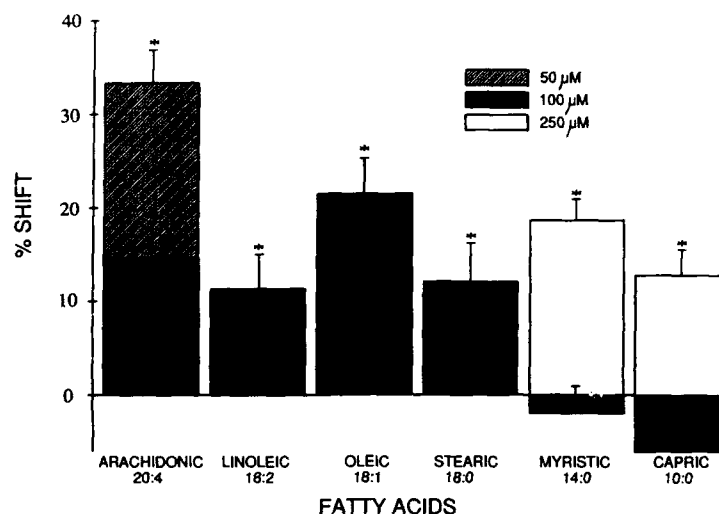


Fig. 4. Washout of fatty acids caused a decrease in ability to generate spikes. Arachidonic acid (50 μ M, 100 μ M), linoleic acid (100 μ M), oleic acid (100 μ M), myristic acid (250 μ M) and capric acid (250 μ M) significantly shifted the relationship between population PSP size and population spike amplitude to the right. All changes are calculated in reference to the appropriate solvent or time control.

control period (squares, single line). In contrast, with the solvent controls, the curve shifted in the opposite direction with time, reflecting a gradual increase in E/S coupling in the absence of any exogenous fatty acids. The fatty acids, therefore, are actually more effective than would appear from Fig. 3.

Some of the fatty acids also increased the maximal population spike amplitude as seen in Fig. 3. Growth of the population spike was also seen with the solvents and time controls. Yet myristic acid (100 and 250 μ M) elicited an increase in maximal amplitude beyond that predicted from the DMSO solvent data, both during exposure and following washout. Similarly, 50 μ M arachidonic acid, 100 μ M linoleic acid and 100 μ M stearic acid increased the maximal population spike significantly greater than with the ethanol controls during the wash period.

Increased population spike

Both synaptic efficacy and E/S coupling contribute to the amplitude of the population spike at a single stimulus strength. Despite the observation that most of the fatty acids did not significantly alter either of the component curves during fatty acid exposure, many did significantly increase the population spike. Since the population spike also grew with the solvent controls, all increases were referenced to the appropriate control values. Only arachidonic acid (50 μ M), stearic acid (100 μ M) and myristic acid (100 μ M) did not significantly increase the population spike. During the 30-min exposure to the fatty acids, the increase in the population spike ranged from 11% with 100 μ M arachidonic acid to 22% with 100 μ M oleic and linoleic acids, relative to the solvent controls.

With a 30-min washout, myristic and capric acids (100 and 250 μ M) showed an additional increase in

the population spike. Following washout, only arachidonic acid and stearic acid did not show significant potentiation of population spike amplitude. The largest potentiation was seen with 250 μ M myristic acid which increased 32% more than the DMSO control. The effects of 100 μ M oleic acid on the relationship between afferent volley and the population spike amplitude are illustrated in Fig. 5. The curve was enhanced during exposure to the fatty acid and the potentiation continued following washout. The increase in population spike amplitude with oleic acid treatment was significantly greater than that seen with the ethanol solvent alone. The time-course of changes in amplitude of the population spike and the population PSP with application of 250 μ M capric acid are shown in Fig. 6. As described above, the population spike increased quickly following exposure to the fatty acid while the population PSP increased more slowly. Following removal of the fatty acid, the population spike continued to increase slightly but the population PSP increased more dramatically. The effects persisted for the duration of the recording period (1 h of wash). The change in amplitude of the population spike during the exposure could result from an increase in synaptic potential and/or an increase in E/S coupling, although neither effect alone was statistically significant. With washout, the synaptic potential was increased disproportionately to the increase in the population spike. This is likely to be a consequence of the decrease in E/S coupling discussed above.

DISCUSSION

A number of fatty acids have been found to produce electrophysiological effects in hippocampal slices. Although each fatty acid was unique, a general pattern was evident. With exposure to the fatty acid

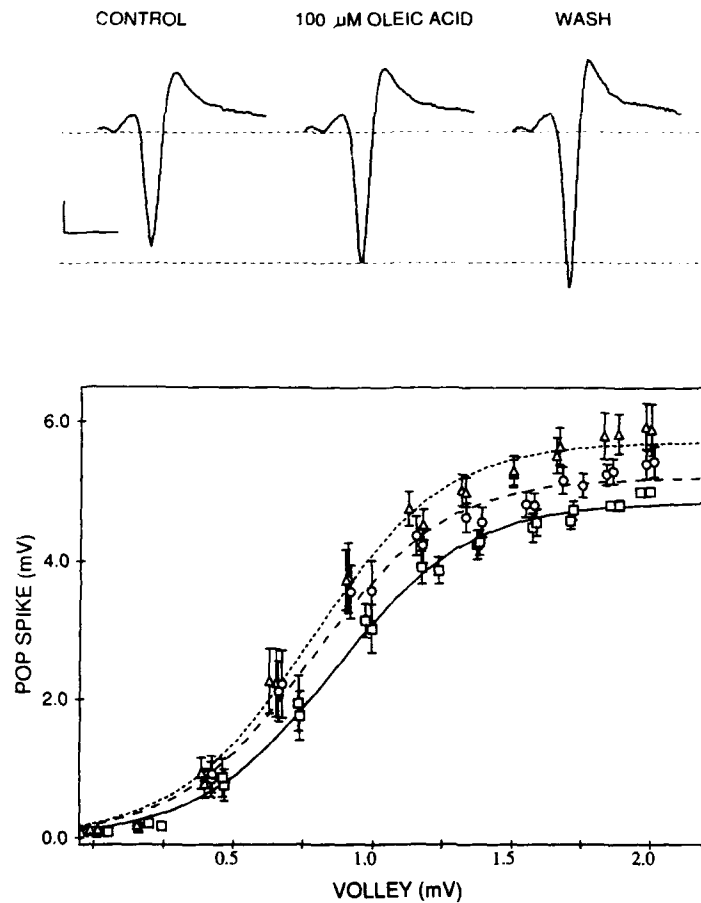


Fig. 5. Exposure to 100 μ M oleic acid increased population spike amplitude. Traces show sample population spikes from a slice exposed to 100 μ M oleic acid. The population spike increased during application and further increased following washout of the fatty acid. Calibration: 1 mV, 2 ms. Plot of afferent volley vs population spike amplitude shows increase with 30-min exposure and further increase following 30-min of wash. These changes exceeded those seen with the ethanol control. □, single line: control; ○, dashed line: during 30-min application of fatty acid; △, dotted line: 30-min wash with normal solution. Curves from five slices were averaged.

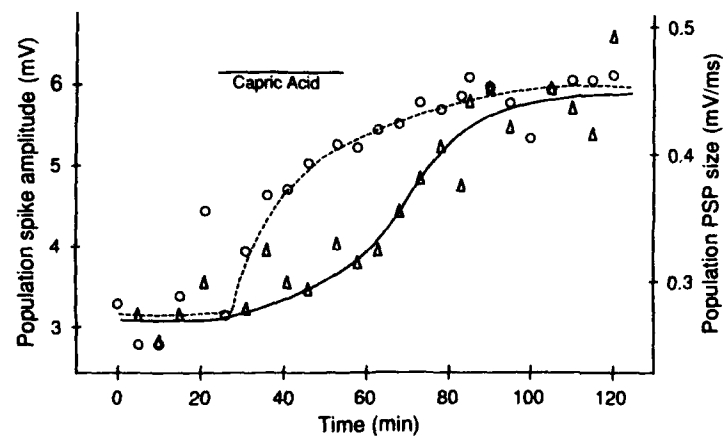


Fig. 6. Time-course of increase in population spike amplitude (○) and population PSP size (△) with exposure to 250 μ M capric acid in a representative experiment. Synaptic potentials increased slightly during exposure to capric acid and showed greater increase with wash. Population spikes showed substantial increase during initial exposure to capric acid. With washout, the population spike did not increase proportionately with the increase in the synaptic response.

the population spike increased. The synaptic potential and E/S coupling were minimally affected during this time. With washout of the fatty acid, the synaptic response potentiated. The population spike frequently continued to increase with wash but not to the extent expected from the rise in the synaptic potential. E/S coupling was impaired. These data suggest that a number of cellular processes are sensitive to fatty acids.

Oleic acid and arachidonic acid have been shown to enhance LTP in the hippocampus.^{14,16,38} Linden *et al.*^{14,16} suggested that these actions resulted from activation of protein kinase C. Oleic acid was more effective than arachidonic acid both to activate protein kinase C and to enhance LTP.¹⁴ Stearic acid, which does not activate protein kinase C^{15,18} did not have this effect.¹⁴ Similarly, in the present study, oleic acid was more effective than arachidonic acid in potentiating the synaptic response while stearic acid was ineffective. Although myristic acid, but not capric acid, was reported to activate protein kinase C,¹⁸ both were found to be equally effective at increasing the synaptic response. The fatty acid actions on spike generation do not follow the same pattern of effectiveness and probably do not result from this mechanism. Increased inositol phosphates and the consequent rise in intracellular calcium has also been proposed as a mechanism for arachidonic acid-induced LTP enhancement.¹⁷ However, oleic acid cannot substitute for arachidonic acid in activation of phosphoinositide turnover.^{17,19} This observation makes it an unlikely mechanism for any of the electrophysiological actions described here.

Takenaka *et al.*^{30,31} have examined the actions of a number of fatty acids on currents in squid giant axon. They found that sodium current activation parameter (*m*) was shifted in the depolarized direction with application of fatty acids with at least eight carbons³⁰ and that the long-chain fatty acids such as arachidonic acid were more effective than the medium-chain fatty acids.³¹ From the chemical structures, Takenaka *et al.*³¹ hypothesized that the fatty acids all have a similar action to perturb the membrane near the "plastic" region of the sodium channel. A similar disruption of the membrane by the fatty acids could alter hippocampal electrophysiology. In fact, Crews *et al.*¹⁰ have shown that alteration of membrane fluidity by addition of cholesterol reversibly reduced the cholinergic response of hippocampal pyramidal cells. Fatty acid insertion into the membrane and modification of local channel domains cannot be excluded as a mechanism for some of the fatty acid actions described here.

Metabolites of arachidonic acid have been suggested as the effective agents in potentiation of orthodromic responses in the hippocampus.^{6,11,37} The

lipoxygenase inhibitor, nordihydroguaiaretic acid, prevented arachidonic acid-induced synaptic potentiation¹¹ but not the enhancement of LTP.³⁸ The actions of arachidonic acid are not blocked by indomethacin, a cyclooxygenase inhibitor.¹¹ The lipoxygenase metabolites, (12)-hydroperoxyeicosatetraenoic acid (12-HPETE)¹¹ and hepxilin A₃⁶ mimicked the synaptic potentiation by arachidonic acid. However, since most of the fatty acids used in the present study are not substrates for either lipoxygenase or cyclooxygenase, a metabolic product is an unlikely mediator of the present results.

Fatty acids may have differential effects when made available intracellularly vs extracellularly. In muscle myocytes, intracellular oleic acid and arachidonic acid increased inward current while extracellular arachidonic acid decreased the inward current.³⁶ It is interesting to speculate that the changes in excitability in the hippocampal slice with exposure and wash may reflect a differential distribution of the fatty acid. This possibility needs to be considered when extrapolating data to pathological states where fatty acids may be expected to accumulate to a greater extent intracellularly than extracellularly.

Fatty acids are released from cells following exposure to free radicals.^{7,8,27} Previous studies in our laboratory have shown that gamma radiation³³ and peroxide-induced free radical damage²¹ cause a decrease in both synaptic efficacy and spike generation. With X-radiation at a low dose rate (1.5 Gy/min) where lipid peroxidation may predominate as a mechanism of cell damage,²⁶ synaptic efficacy is increased. The curve relating the ability of the population PSP to generate a population spike is shifted to the right. In addition, the maximal population spike amplitude is increased. In short, the electrophysiological consequences of X-radiation look very much like exposure and subsequent washout of fatty acids. We postulate that the release of fatty acids contributes to the electrophysiological effects of X-radiation exposure. Free radical-induced release of fatty acids, in combination with additional factors, probably contribute to peroxide and gamma radiation damage as well. The results of the present study support the possibility that free fatty acids contribute to the electrophysiological changes that occur in pathological states such as ischemia.

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