Fatty Acid Ethyl Esters, Nonoxidative Metabolites of Ethanol, Accelerate the Kinetics of Activation of the Human Brain Delayed Rectifier K^+ Channel, Kv1.1*  

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Herein we demonstrate that the major metabolites of ethanol in neural tissues, fatty acid ethyl esters, dramatically accelerate the kinetics of the voltage-induced activation of the human brain delayed rectifier potassium channel, Kv1.1. Specifically, the external application of ethyl oleate (20 μM) to Sf9 cells expressing the recombinant Kv1.1 channel resulted in a decrease in the rise times of the macroscopic current (e.g. from 51.7 ± 13.1 to 12.8 ± 3.0 ms at 0 mV for 10–90% rise times) and a 10-mV hyperpolarizing shift (at 0 mV) in the voltage dependence of channel activation. These effects were dose-dependent (half maximal effect at 7 μM), saturable and specific (i.e. fatty acid methyl esters were without effect). Although application of either ethanol or oleic acid alone did not result in alterations of the activation kinetics, the concomitant application of ethanol and oleic acid reproduced the effects of fatty acid ethyl esters with a temporal course which paralleled the intracellular accumulation of fatty acid ethyl esters in Sf9 cells. Moreover, application of fatty acid ethyl esters (but not ethanol) to rat hippocampal cells in culture produced similar effects on hippocampal delayed rectifier currents. Collectively, these results demonstrate that pathophysiologically relevant concentrations of metabolites of ethanol, fatty acid ethyl esters, modulate the function of a prototypic neuronal ion channel and thus likely contribute to the pathophysiologic sequelae of ethanol abuse in excitable tissues.

Elucidation of the biochemical mechanisms underlying the effects of ethanol in electrically excitable tissues has been a long standing goal in neurochemistry (e.g. Refs. 1–3). Due to the structural simplicity of ethanol, the difficulties in identifying the precise biochemical mechanisms responsible for ethanol-induced alterations in electrophysiologic function have represented a fundamental paradox in neuropharmacology. Although multiple effects of ethanol on ion channel function have been demonstrated, consistent alterations in ion channel properties at pathophysiologically relevant concentrations of ethanol (i.e. 0.01–0.1% (2–20 mM)) have not been forthcoming (4–7). The discordance between the amounts of ethanol necessary to modulate ion channel function in isolated cells and that necessary for perturbation of electrophysiologic function in intact neural structures represents an unresolved and highly controversial issue in the pathobiology of alcohol.  

Fatty acid ethyl esters are the major nonoxidative metabolite of ethanol in humans and represent the predominant ethanol metabolite in human brain after alcohol ingestion (8–10). Moreover, detailed examination of fatty acid ethyl ester synthesis in distinct anatomic loci in brain has demonstrated a high correlation between ethanol-induced pathology and the metabolic rates of fatty acid ethyl ester production (11). Since recent studies have identified the electrophysiologic effects of nonesterified fatty acids on ion channels (12–17), we hypothesized that some of the electrophysiologic effects of ethanol could result from the interaction of ethanol metabolites (i.e. fatty acid ethyl esters) with ion channels. Accordingly, we examined the electrophysiologic effects of pathophysiologically relevant amounts of fatty acid ethyl esters (i.e. that found in humans after alcohol consumption (18)) on Sf9 cells expressing the recombinant neuronal delayed rectifier K^+ channel, Kv1.1, which has previously been shown to be “insensitive” to high concentrations of ethanol (5). We now report that fatty acid ethyl esters induce a profound increase in the kinetics of the voltage-induced activation of the Kv1.1 channel, that the observed effects are saturable and specific, that the effects of ethanol on Kv1.1 channel function are tightly correlated with the rates of cellular synthesis of fatty acid ethyl esters, and that fatty acid ethyl esters, but not ethanol, result in electrophysiologic alterations of the delayed rectifier potassium currents in intact hippocampal cells.

MATERIALS AND METHODS

Expression of Kv1.1—The purified Kv1.1 recombinant virus, which was kindly provided by Dr. A. Kamb (19, 20), was subsequently amplified and titered to a concentration of 10^6 plaque-forming units/ml. Spodoptera frugiperda (Sf9) cells were infected with the Kv1.1 virus at a multiplicity of infection = 1.7 for 72 h utilizing standard methodology (21).

Electrophysiologic Measurements in Sf9 Cells—Whole-cell voltage clamp recordings of Sf9 cells were performed utilizing standard techniques as described previously (17, 22). Data were acquired, and activation time constants were determined through best fit analysis of monoexponential equations corresponding to 10–90% rise times. The indicated fatty acid ethyl esters were dissolved in dimethyl sulfoxide at a concentration of 20 μM and diluted into extracellular solution to a final concentration of 20 μM (unless otherwise stated), with a final dimethyl sulfoxide concentration of less than 0.1%. In hippocampal cells (kindly provided by C. Zorumski (23)), we minimized A-type K^+ current (I_A) through a standard voltage-clamp paradigm, which included a prepulse of 150 ms to −20 mV to inactivate I_A before the depolarizations to evoke delayed rectifier currents (I_K) (24, 25). Ca^2+-dependent currents were minimized by the inclusion of 1,2-bis(2-aminophenoxy)ethane-N,N,N,N-tetraacetic acid in the recording solutions, while Na^+ currents were either blocked by tetrodotoxin or separated from I_K during the prepulse described above.

Quantification of Ethyl Ester Synthesis in Sf9 Cells—The conversion of oleic acid to ethyl oleate by Sf9 cells was examined by incubating Sf9 cells with 60 μCi of 9,10-^3H)oleic acid (20 μM) and ethanol (20 mM) for 0–15 min at 22 °C. Acetone extracts were evaporated under a stream of nitrogen, and fatty acid ethyl esters were purified by TLC and quantified by scintillation spectrometry as described previously (26).
Ethyl Esters Modulate K+ Channel Function

RESULTS

Fatty Acid Ethyl Ester-induced Alterations in Kv1.1 Macroscopic Current—Superfusion of cells with ethyl oleate or ethyl arachidonate (20 μM) resulted in a profound increase in the rate of the voltage-dependent activation of the macroscopic current carried by Kv1.1 channels expressed in SF9 cells (Fig. 1A). Activation was not manifest when either ethanol (up to 200 mM) or oleic acid (up to 50 μM) were individually applied (Fig. 1, B and C, respectively). Moreover, electrophysiologic alterations in the macroscopic current were manifest when oleic acid and ethanol were concomitantly applied (Fig. 1D), and the magnitude of electrophysiologic alterations was correlated with the accumulation of endogenously synthesized fatty acid ethyl esters (vide infra). Endogenously Generated Fatty Acid Ethyl Esters Mediate Alterations in Kv1.1 Macroscopic Current—Perfusion of SF9 cells with radiolabeled oleic acid and ethanol resulted in the production of ethyl oleate at a rate of 1.4 pmol/10^6 cells/min. Alterations in the kinetics of the macroscopic current of Kv1.1 were first visualized 3 min postapplication corresponding to the presence of measurable synthesis of fatty acid ethyl esters (Fig. 2, A and B). With time, the magnitude of electrophysiologic alterations increased correlating with the accumulation of fatty acid ethyl ester mass. In contrast, no alterations in the macroscopic current were manifest with ethanol or oleic acid alone even after perfusion for 15 min (data not shown).

Kinetic Characterization and Structure-Activity Relationships of Fatty Acid Ester-mediated Alterations in Kv1.1 Macroscopic Current—The effects of ethyl oleate on the monoeponential time constant of activation (τact) were dose-dependent and saturable with half-maximal activation manifest at 7 μM ethyl oleate (Fig. 3A). Although the increase in the rate of activation of Kv1.1 by ethyl arachidonate was not accompanied by a substantial increase in the steady-state macroscopic current, application of ethyl arachidonate did result in a 10–20 mV hyperpolarizing shift in the current-voltage relationship (Figs. 1A and 3B). Decreases in the time constant of activation were seen over a range of depolarizing voltages from −10 mV to +20 mV (Fig. 1A). Application of fatty acid ethyl esters resulted in a dramatic decrease in the calculated 10–90% rise times of the voltage-dependent activation of the Kv1.1 macroscopic current (Fig. 3C). To examine the structure-activity relationships underlying these effects, the chain lengths of the ester moiety and the length and degree of unsaturation of the fatty acyl chain were varied. Remarkably, fatty acid methyl esters did not induce any significant kinetic alterations in macroscopic current development even at concentrations up to 50 μM, suggesting that nonspecific membrane interactions were not responsible for the electrophysiologic alterations seen with ethyl esters. In contrast, lengthening the ester linkage (e.g. propyl and butyl) mimicked the ethyl ester effects (Fig. 3D). Furthermore, the nature of the aliphatic group did not contribute to the observed electrophysiologic effects, since ethyl esters comprised of 16:0, 18:1, 20:4, and 22:6 fatty acyl chains and propyl and butyl esters comprised of 18:1 and 20:4 fatty acyl chains produced identical alterations in macroscopic current development (Fig. 3E).

Fatty Acid Ethyl Ester-induced Alterations in Hippocampal K+ Current—To demonstrate that the observed electrophysiologic alterations on Kv1.1 function produced by fatty acid ethyl esters in the context of SF9 cells were also an inherent and physiologic property of channel function in a natural context, we applied ethyl arachidonate to hippocampal cells grown in microisland cultures. Since hippocampal cells contain many structurally and functionally distinct K+ channels (e.g. delayed rectifier and A-type channels), we employed a prepulsing voltage paradigm to deconvolute the complexities inherent in voltage-current relationships generated by competing ion channels. To observe the effects of ethyl esters on hippocampal delayed rectifiers, cells were prepulsed for 150 ms at −20 mV to inactivate the A-type channels (24, 25). Application of ethanol (up to 200 mM) failed to induce any measurable alterations in the K+ macroscopic current carried by delayed rectifiers (Fig. 4A). In contrast, application of ethyl arachidonate resulted in similar alterations in voltage-dependent potassium current kinetics as those manifest in SF9 cells (e.g. an increase in the kinetics of activation) (Fig. 4B). Moreover, quantification of the fatty acid ethyl ester-induced alterations in 10–90% rise times of the delayed rectifier current in hippocampal cells demon-
strated a decrease from 27.3 ± 5.5 to 9.7 ± 2.0 ms at 110 mV (p, 0.025). As anticipated, application of fatty acid methyl esters to hippocampal cells did not have electrophysiologic effects, while application of other fatty acid ethyl esters (e.g., ethyloleate) and fatty acid propyl esters showed characteristic modulation of hippocampal delayed rectifier K⁺ current (data not shown).

DISCUSSION

Typical neuronal action potentials last between 2 and 10 ms, and their amplitude and duration are intimately related to the kinetics of participant voltage-dependent K⁺ channels (25, 27–30). In brain, the Kv1.1 ion channel is the predominant delayed rectifier in axons (31–33) and presynaptic terminals (32) and is prominent in specialized neuronal subtypes that actively synthesize fatty acid ethyl esters and are the targets of alcohol toxicity (e.g., hippocampal cells (32, 34, 35)). The results presented herein identify profound alterations in the kinetics of the developed macroscopic current carried by Kv1.1 induced by metabolites of ethanol, fatty acid ethyl esters, while ethanol and oleic acid alone are without effect. Accordingly, alterations in the kinetics of activation occurred in a critical time frame during which the extent and extant of synaptic transmission is modulated by the strength and duration of the depolarizing impulse. Moreover, the substantial hyperpolarizing shift in the voltage dependence of activation is anticipated to promote synaptic depression through enhanced repolarization, which is consistent with the well-known depressant effects of alcohol on target neurons (1, 36, 37). Indeed, prior reports describe a hyperpolarization of hippocampal neurons by ethanol and propose that an increase in potassium conductance, as demonstrated herein, represents the underlying mechanism (38, 39).

In independent seminal studies, both Singer and Clapham demonstrated the profound effects of nonesterified fatty acids in microisland cultures were performed utilizing a prepulse from a holding potential of −80 mV to −20 mV (not pictured) followed by depolarizing voltage steps to the indicated potentials as described under “Materials and Methods.” A, overlay of Kv1.1 macroscopic current traces before (dotted line) and after application (solid line) of ethanol (20 mM). B, overlay of Kv1.1 macroscopic current traces before (dotted line) and after application (solid line) of ethyl arachidonate (20 μM). C, quantification of alterations in 10–90% rise times of the macroscopic current carried by hippocampal delayed rectifiers at +10 mV induced by ethyl arachidonate (20 μM). Data represent mean ± S.E. of three independent experiments (p < 0.025).
inactivation of the macroscopic current carried by the Kv1.1 channel. Second, precise stereoelectrochemical determinants of the aliphatic constituent are necessary for the appropriate interaction of arachidonic acid with the Kv1.1 channel (17). Third, N-terminal mutants of the Kv1.1 channel demonstrate substantial alterations in the voltage dependence of arachidonic acid-induced inactivation, whereas activation by fatty acid ethyl esters in these N-terminal mutants is indistinguishable from that manifest by wild type.\(^1\) Collectively, these data suggest that fatty acid ethyl esters and arachidonic acid interact with the Kv1.1 channel at independent sites.

Structure activity analyses demonstrated that alterations in the aliphatic constituent of the fatty acid ethyl ester do not change electrophysiologic effects and that ester linkages \(\geq 2\) carbons are necessary for electrophysiologic perturbations. Accordingly, it seems likely that the function of the ester linkage is to facilitate reinsertion of the ester moiety into the membrane interface leading to its subsequent interaction with the ion channel. In this paradigm, after membrane insertion the fatty acid ethyl ester diffuses laterally to interact with the ion channel and through specific interactions of the carboxyl ester linkage with the channel can modulate its voltage-dependent conformational transitions. The novel biologic theme elaborated by these observations is the demonstration that a drug (i.e. ethanol) can, through enzyme-catalyzed ligation to an endogenous delivery agent (i.e. fatty acid), participate in host-guest interactions in biologic membranes with ion channels.

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