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Short communication

Suppression of evoked IPSPs by arachidonic acid and prostaglandin $F_{2\alpha}$

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Abstract

Arachidonic acid (AA) and certain prostaglandins appear to antagonize $GABA_A$ receptors in synaptoneurosomes [18]. We report here that perfusing hippocampal slices with AA or prostaglandin $F_{2\alpha}$ diminishes evoked IPSP conductance and increases CA1 pyramidal cell input resistance. The effects of the two compounds were similar, though not identical, in time course, magnitude, and response to washout. These findings suggest that high levels of AA and its metabolites may bias neurons towards excitation.

Keywords: CA1; Eicosanoid; Epilepsy; Excitotoxicity; Hippocampus; Input resistance

While characteristic patterns of nerve cell loss have been described for certain epilepsies [13], the actual mechanism of cell death in these disorders remains unclear. According to the excitotoxicity hypothesis [5,10,15] seizure activity releases large quantities of excitatory amino acids (EAAs), particularly glutamate, which promote prolonged neuronal depolarization and lead to elevated intracellular calcium. Dysregulation of calcium homeostasis produces uncontrolled activation of intracellular proteases, nucleases, and lipases leading to cell injury and, eventually, cell death.

Arachidonic acid (AA) and its metabolites are, like calcium, normal signalling molecules which can produce pathological changes at high concentrations [11,16,19]. Seizures, head trauma, hypoglycemia, and ischemia elevate intracerebral levels of eicosanoids (AA, prostaglandins, thromboxanes, and leukotrienes) [1,14,18,20]. NMDA receptor-mediated calcium influx, thought to be integral to some forms of excitotoxicity [5,10], activates PLA_2 which splits AA out of the plasma membrane [17,18,21]. Metabolism of AA produces vasoactive compounds (e.g. LTC_4) which may alter cerebrovascular resistance and permeability leading to vasogenic edema [2]. Furthermore, AA breakdown by cyclooxygenase, lipoxygenase, or microsomal cytochrome P_{450} produces oxygen free radicals which can cause lipid peroxidation, protein denaturation, and DNA damage [12,21].

Another role for eicosanoids in excitotoxicity is suggested by the recent finding that AA and certain prostaglandins antagonize muscimol-induced Cl^- flux into synaptoneurosomes, presumably by blocking $GABA_A$ receptors [18]. Blockade of $GABA_A$ receptors during episodes of hyperexcitability would exacerbate excitotoxic effects and promote continued synaptic bombardment. We set out to determine the effect of exogenous eicosanoids on inhibition in a partially intact neural system: the hippocampal slice. We found that, in hippocampal pyramidal cells, AA and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) application significantly suppressed evoked IPSP conductance and increased input resistance.

Female Sprague–Dawley rats (150–250 g) were sacrificed by decapitation and the brain removed, cooled with ice cold oxygenated artificial cerebrospinal fluid (ACSF: NaCl 124 mM, $NaHCO_3$ 26 mM, NaH_2PO_4 1.25 mM, KCl 3 mM, $MgSO_4$ 2.0 mM, $CaCl_2$ 2 mM, and glucose 10 mM), blocked, and glued to a vibratome stage with cyanoacrylate. Horizontal hippocampal slices were cut at 400 μm and submerged in oxygenated room temperature ACSF until needed for recording.

Slices were placed in a low volume submersion chamber, held under a fine gold wire mesh, and perfused (2.5 ml/min) with 29–31°C oxygenated ACSF. Sharp glass electrodes (60–110 MΩ) filled with 4 M potassium acetate were lowered into the CA1 pyramidal cell layer. The

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electrode was advanced to obtain stable intracellular impalements (stable resting membrane potential without holding current, few spontaneous action potentials), and postsynaptic potentials (PSPs) were then evoked with stratum oriens/alveus stimulation (fine insulated tungsten monopolar electrode) located 200–800 μm from the recording electrode. Measurements were made on a component of the PSP with the known characterisitics of the 'fast' ($GABA_A$) IPSP in hippocampal pyramidal cells —i.e., peak latency at 8 to 15 ms after the stimulus and reversal potential of −65 to −70 mV (Fig. 1A) These IPSPs were examined at a series of hyperpolarized and depolarized membrane potentials (relative to rest) obtained by tonic current injections into the impaled neuron; IPSPs were evoked at each potential (at a frequency of 0.1 Hz) to allow calculation of IPSP reversal potential as well as IPSP conductance (see below). Following collection of baseline data, the normal perfusion fluid was switched to one containing 150 μM AA or 75 μM $PGF_{2\alpha}$ dissolved in oxygenated ACSF (the 'test solution'). In the following discussion, 'baseline' will refer to data collected in normal ACSF prior to switching to the test solution while 'test solution' will refer to data collected after switching to ACSF containing eicosanoids. Control experiments were performed in exactly the same manner as described above, with the test solution containing only normal ACSF. In both cases, after at least 15 min in the test solution, the fluid input was switched back to the original ACSF.

Voltage and current signals were amplified and digitized on-line at 0.5 kHz. For each cell, we measured the following every 2 to 3 min: input resistance, IPSP reversal potential, IPSP conductance, and latency to IPSP peak. IPSP peak latency was determined during the baseline period and was used for all subsequent measurements for a

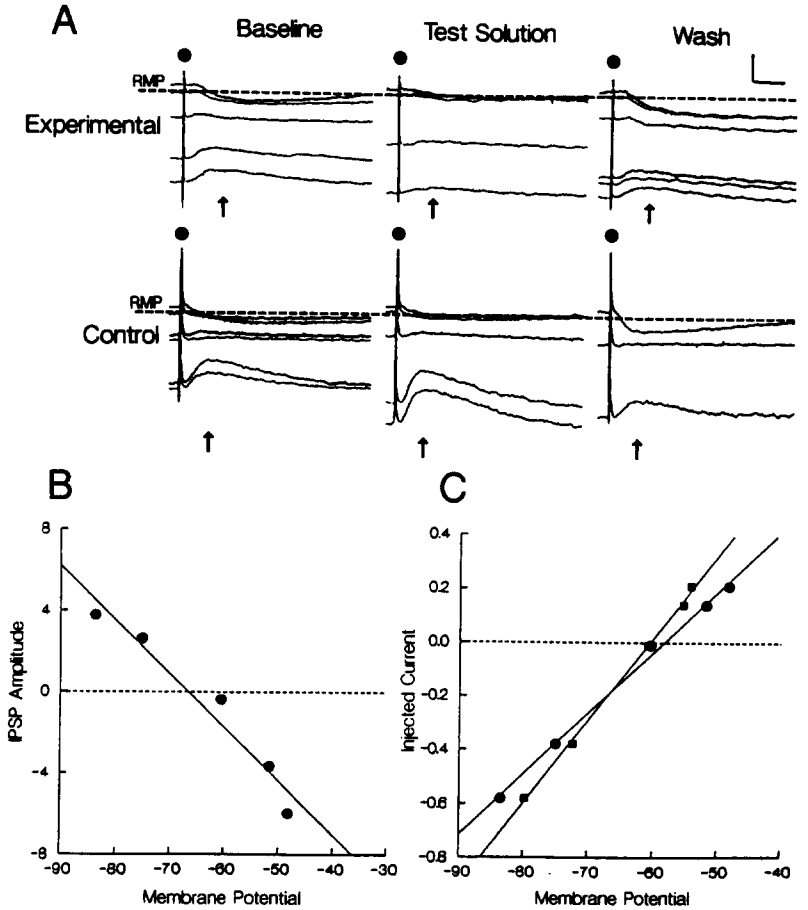

Fig. 1. A: IPSP reversal series from the three different epochs for a control cell and an experimental cell perfused with 150 μM AA. Under each condition ('baseline', 'test solution', and 'wash') the synaptic response was recorded with the cell held at several different membrane potentials by injecting dc current intracellularly. Sample traces are shown at progressively more negative potentials, illustrating the reversal of the 'fast' IPSP (arrow) from a hyperpolarizing event seen at resting membrane potential (dashed line) to a depolarizing event. The successful washout shown here was unusual. Dots mark stimulation artifacts and arrows show where IPSP conductance was measured. (calibration bar: 10 mV and 10 ms). B: example of reversal potential calculation using data from the experimental baseline reversal series in 'A'. IPSP amplitude (measured at arrows) vs. membrane potential plot reveals a reversal potential of −67 mV. C: example of IPSP conductance and input resistance calculation. Injected current vs. membrane potential plot comparing resting potential (circles; measured 40 ms before the stimulus artifact) and IPSP peak potential (squares; measured at arrows). Subtracting the resting slope conductance (22.7 nS) from the total conductance at IPSP peak (30.8 nS) reveals the IPSP conductance (8.1 nS). The inverse of the resting slope conductance is the input resistance (44.1 MΩ).

given cell. IPSP reversal potential was determined by plotting peak IPSP amplitude against membrane potential (altered by injection of d.c. holding current) and taking the *x intercept* of a least squares regression line (See Fig. 1B). IPSP conductance was calculated by subtracting the cell's input conductance 40 ms prior to stimulation from the conductance at IPSP peak (See Fig. 1C). Input conductance was the slope of a least squares regression line through a plot of injected d.c. holding current vs. membrane potential 40 ms before the stimulus. Conductance at IPSP peak was calculated similarly except the membrane potential was measured at IPSP peak. Input resistance was taken to be the inverse of the input conductance.

Since baseline IPSP conductance and input resistance varied among cells, these measurements were standardized for each cell by dividing conductance or resistance at each time point by the mean value for that parameter during the baseline epoch. A standardized IPSP conductance of 0.5 during test solution perfusion then, would indicate a reduction of IPSP conductance by half relative to the conductance measured at baseline. These standardized data from each cell were then combined and grouped into 5 min bins for statistical analysis.

In all, data from 25 CA1 cells (9 control, 10 perfused with 150 μM AA, and 6 perfused with 75 μM $PGF_{2\alpha}$), each from a different slice, were analyzed. Mean baseline input resistance, IPSP reversal potential, resting membrane potential, and IPSP conductance did not differ significantly between experimental and control cells (Table 1). Since the distance of a cell from the perfused surface might alter the effect of the drug solution, cell depth in the slice was also compared and found not to differ.

Table 1
Initial equivalence of groups

	Control	Experimental
Input resistance (MΩ)	52.92 ± 4.48	46.07 ± 5.21
Resting membrane potential (mV)	−63.5 ± 1.8	−63.8 ± 1.2
IPSP conductance (nS)	7.16 ± 2.02	10.56 ± 3.40
IPSP reversal potential (mV)	−67.67 ± 1.83	−69.64 ± 1.95
Depth (μm)	105 ± 22	139 ± 14

Stimulation in stratum oriens/alveus produced either a short latency IPSP or a small EPSP followed by a larger IPSP. Although the IPSP was not pharmacologically isolated, the reversal potential and peak latency both suggest that this response was a fast IPSP. Fig. 1 shows a reversal series from each of the three epochs for an experimental cell and a control cell as well as an example of data

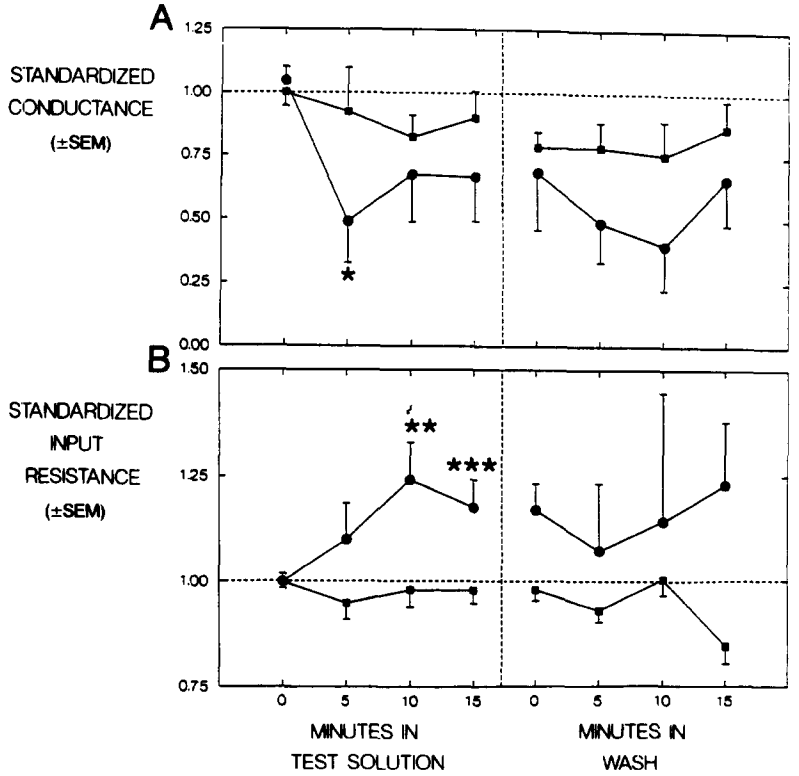

Fig. 2. Standardized IPSP conductance (A) or input resistance (B) for experimental (150 μM AA; circles) and control (squares) conditions plotted against time in test solution (left side) or against time in wash (right side). Time zero on the left side represents the mean value of the parameter during the baseline epoch while zero on the right side represents the mean value of the parameter for the ten minutes immediately preceding the wash epoch. (A) * = significantly different from baseline experimental value (Tukey HSD, $P = 0.031$). (B) * * = significantly different from every control value as well as the experimental value at baseline (Tukey HSD, $P<0.002$). * * * = significantly different from control value at 5 min (Tukey HSD, $P = 0.017$). Abscissa applies to both A and B.

analysis. Perfusion with 150 μM AA significantly diminished standardized IPSP conductance over time (ANOVA, $P = 0.008$) as well as relative to perfusion with control ACSF (ANOVA, $P = 0.025$) (Fig. 2A, left side). This decrease in conductance was greatest after 5 min in AA. Although simply switching to control ACSF appeared to affect conductance somewhat, the effect was small relative to the effect of arachidonate and was not statistically significant. Switching the perfusion medium back to normal ACSF failed to produce a consistent return to baseline.

In addition to decreasing IPSP conductance, perfusion with AA significantly increased input resistance over time (ANOVA, $P = 0.025$) as well as relative to perfusion with control ACSF (ANOVA, $P < 0.000005$) (Fig. 2B, left side). This effect was maximal after 10 min in arachidonate. Switching the perfusing medium to control ACSF did not significantly affect input resistance. After switching back to normal ACSF, standardized input resistance remained statistically different (ANOVA, $P = 0.003$) but did not change significantly over time (Fig. 2B, right side).

Perfusion with 75 μM $PGF_{2\alpha}$ affected standardized IPSP conductance and input resistance similarly to 150 μM AA (Fig. 3). Conductance was diminished significantly over time (ANOVA, $P = 0.008$) as well as relative to perfusion with control ACSF (ANOVA, $P = 0.002$). Unlike perfusion with AA, standardized IPSP conductance did not return towards baseline but, like AA, failed to demonstrate an effect of washout. Perfusion with 75 μM $PGF_{2\alpha}$ increased input resistance relative to perfusion with control ACSF (ANOVA, $P = 0.004$) and this effect did not return to baseline —even after washes of up to 90 min. During washout, standardized IPSP conductance and input resistance remained statistically different from control values (ANOVA, $P = 0.007$ and $P > 0.001$ respectively) but did not change significantly over time.

Eicosanoids have been reported to modulate several ionic currents [8,9,11,16,19,22,23], producing either a net excitatory or inhibitory effect. In the present study, we have found preliminary evidence of an eicosanoid-mediated antagonism of evoked fast IPSP conductance as well as a concomitant increase in CA1 input resistance. Both effects followed a similar time course, peaking between 5 and 10 min and failing to reverse convincingly. Such actions of AA or $PGF_{2\alpha}$ would bias neurons towards increased excitability. Arachidonate may itself interact directly with channels and transporters, or it may act through its metabolites: leukotrienes and prostaglandins. We have shown similar effects for both AA and $PGF_{2\alpha}$, but our data cannot address the question of whether or not AA is acting through its metabolites.

The most parsimonious explanation of our results would be antagonism of evoked and spontaneous fast IPSPs by AA and $PGF_{2\alpha}$ which would increase input resistance by decreasing the background conductance. Along these lines, Alger and Nicoll [3] reported that application of the $GABA_A$ antagonist bicuculline to hippocampal slices increased CA1 input resistance. In contrast to such a proposed post-synaptic effect, 10 μM AA has been shown to

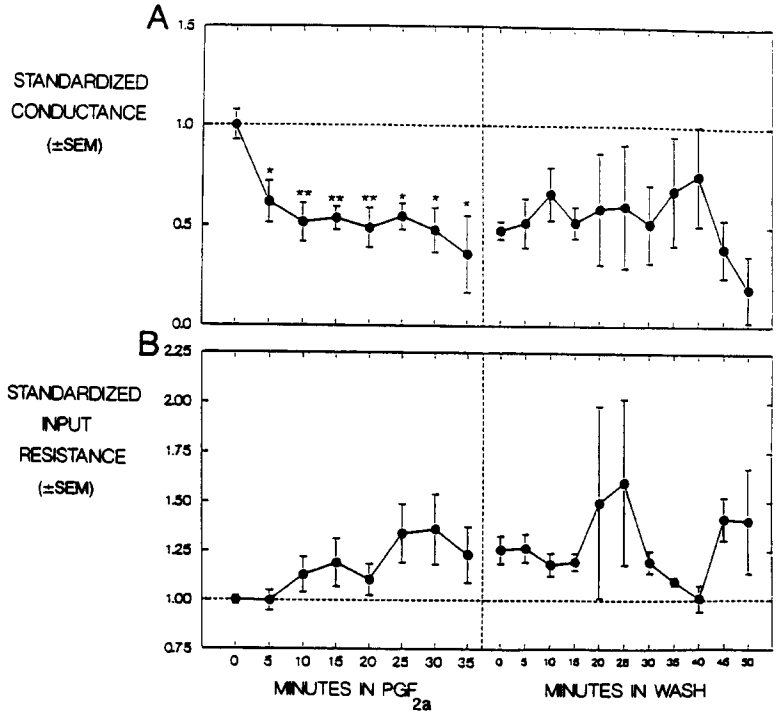

Fig. 3. Standardized IPSP conductance (A) or input resistance (B) for 7 cells plotted against time in 75 μM $PGF_{2\alpha}$ (left) or against time in wash (right). (A) * = significantly different from baseline (Tukey HSD, $P < 0.05$). * * = significantly different from baseline (Tukey HSD, $P < 0.01$). Abscissa applies to both A and B.

inhibit pre-synaptic release of GABA from cultured striatal neurons by suppressing voltage-dependent sodium currents [8]. In preliminary experiments however, we found that perfusion with 150 μM AA or 75 μM $PGF_{2\alpha}$ decreased the conductance change associated with focal exogenous application of 5 mM GABA; this result would be consistent with a post-synaptic locus of eicosanoid effect, although not ruling out a possible pre-synaptic action.

In contrast to the results reported here, Carlen and colleagues [4] found that 8–26 μM AA *increased* evoked IPSP amplitude in 4 out of 5 CA1 cells in hippocampal slices without changes in input resistance. This discrepancy between studies could simply reflect the differential effects of different concentrations of AA (8–26 μM vs. 150 μM in our experiments). Furthermore, for the majority of their experiments, Carlen et al. used the methyl ester of AA, which is cleaved into the free acid only after entering the cell (although one cell treated extracellularly with the free acid still showed increased IPSP amplitude). Since treatment with the free acid (as we have done in our experiments) allows the exogenous arachidonate to work either intracellularly or extracellularly, this choice of AA form may also contribute to our differing results.

In addition to a direct effect on IPSPs, it is possible that AA-mediated modulation of another background conductance contributed to the observed changes in input resistance and/or IPSP conductance, especially since the dose of AA used here (150 μM) was well above the EC_{50} previously shown for $GABA_A$ antagonism (29 μM; [18]). Fast sodium and high threshold calcium currents are diminished by eicosanoids in certain neurons [8,11,16]; such AA-mediated effects could reduce the IPSP conductance by affecting transmitter release. Several potassium currents are also augmented by AA [9,16,23]; indeed, somatostatin's enhancement of I_m in CA1 pyramidal cells is most likely mediated by a second messenger cascade involving AA [19]. In contrast, investigators have demonstrated a reduction of A-current conductance by AA in bullfrog sympathetic neurons [22]. Although reduced potassium conductance could, in theory, contribute to our observed increase in input resistance, it is unlikely that such a resting membrane effect was mediated by drug actions on a transient conductance such as I_A. It is also not likely to be due to decreased EPSPs, since eicosanoids have been reported to both stimulate release of glutamate as well as suppress glutamate reuptake [6,23].

As in the studies of Schwartz and Yu [18], eicosanoids were not dissolved in a solvent vehicle but were instead dissolved in oxygenated ACSF. Eicosanoid hydrophobicity may have significantly attenuated the amount of drug which reached the impaled cells. This would lead to a relative underestimation of the size of the effect on inhibitory potentials as well as a false elevation of the EC_{50}. Furthermore, the large amount of variance associated with test solution and washout measures may be, in part, due to variable drug delivery.

In the present study, IPSP attenuation by AA peaked at 5 min after drug introduction and then returned towards, but did not reach, baseline. The explanation for the decline of this drug effect is unclear, but may reflect the interaction of AA changes on distinct PSP and resting membrane mechanisms. For example, increased input resistance would amplify synaptic currents, producing a larger apparent conductance at the soma. Drug action that increased input resistance would therefore mask attenuation of the IPSP conductance and, as input resistance increased over time, IPSPs would appear to return towards baseline. However, assuming that AA and $PGF_{2\alpha}$ act identically, the fact that $PGF_{2\alpha}$ did not produce a similar return towards baseline would argue against this hypothesis.

In our preparation, neither the resting slope conductance nor the IPSP conductance returned to baseline values after perfusion with normal ACSF. Although it is not surprising that 15 min of washout was insufficient to completely reverse the drug effect, washes of up to 90 min were similarly ineffective. Beyond the obvious possibility that eicosanoid lipophilicity makes washout difficult, AA may set up long term changes in synaptic properties. For example, AA has been postulated as a possible retrograde messenger in LTP [24]. Furthermore, Drapeau and colleagues reported that simply perfusing hippocampal slices with 200 μM AA either potentiated (12 out of 19 experiments) or depressed (5 out of 19 experiments) the amplitude of CA1 field EPSPs and population spikes [7]. Interestingly, both the potentiation and the depression only began to reverse after 60 to 90 min wash and washout often initially further increased the effect.

The relatively high concentration of AA utilized in this study may bias CA1 cells in an excitatory direction by decreasing inhibition and increasing input resistance. Such effects could have significance for initiating or maintaining the neuronal hyperexcitability characteristic of epilepsy. Furthermore, functional impairment of inhibition by eicosanoids during seizures or ischemia may complement EAA-mediated excitotoxicity and contribute to the demise of 'vulnerable' cells. If this is so, then modulation of eicosanoid metabolism may provide another pharmacological approach to ameliorating excitotoxic neuropathology.

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