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Phospholipase A_2 activation is not required for long-term synaptic depression

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Abstract

Low-frequency synaptic stimulation evokes long-term depression of synaptic strength. One hypothesis is that modification of AMPA receptors by phospholipase A_2 causes long-term depression. A previous study reported bromophenacylbromide, a completely nonselective phospholipase A_2 inhibitor, blocked long-term depression at Schaffer collateral-CA1 synapses in hippocampus. In contrast, I show here that 3-(4-octadecyl)-benzoylacrylic acid (OBAA), a much more potent and selective inhibitor of low and high molecular weight phospholipase A_2, does not block long-term depression at these same synapses, indicating that phospholipase A_2 is not necessary for modifications causing long-term depression.

Keywords: Hippocampus; Long-term depression; Phospholipase A_2

Activity-dependent plasticity includes both long-term potentiation and long-term depression of synaptic strength. Long-term potentiation is elicited by coincidence of presynaptic firing with postsynaptic depolarization, whilst long-term depression is evoked when presynaptic activity occurs while the postsynaptic neuron is relatively inactive (Stanton and Sejnowski, 1989). Increased intracellular $[Ca^{2+}]$ seems to be necessary to induce both long-term potentiation and long-term depression, with large rises necessary for long-term potentiation and smaller increases needed for long-term depression. While much work has examined roles of calcium-activated enzymes in long-term potentiation, much less is known about cellular mechanisms causing long-term depression.

One calcium-activated enzyme proposed to play a role in synaptic plasticity is phospholipase A_2. The induction of long-term potentiation has been reported to be prevented by bromophenacylbromide, a putative inhibitor of phospholipase A_2 (Massicotte et al., 1990). Furthermore, this group has also recently shown a block of the induction of long-term depression by bromophenacylbromide (Fitzpatrick and Baudry, 1994). Unfortunately, bromophenacylbromide is a powerful alkylator that is both nonselective and toxic. Bromophenacylbromide inhibits phospholipase C, protein kinase C and other enzymes in the same concentration range as phospholipase A_2 (see Blackwell and Flower, 1983). Therefore, I reexamined the question of a role for phospholipase A_2 in synaptic plasticity, using a much more potent and selective phospholipase A_2 inhibitor, 3-(4-octadecyl)-benzoylacrylic acid (OBAA; Köhler et al., 1992; BIOMOL Chemicals).

Hippocampal slices (400 μm) were prepared from male Sprague-Dawley rats and maintained in an interface chamber at 33–35°C in artificial cerebrospinal fluid (126 mM NaCl, 5 mM KCl, 1.25 mM NaH_2PO_4, 2 mM $MgCl_2$, 2 mM $CaCl_2$, 26 mM $NaHCO_3$, and 10 mM glucose). Schaffer collateral axons were stimulated, and extracellular e.p.s.p.s recorded in stratum radiatum of area CA1. 5 μM OBAA was bath applied 30–60 min before either low-frequency stimulation to induce long-term depression (LFS; 1 Hz/15 min), or high-frequency theta stimulation to evoke long-term potentiation (HFS; 100 Hz/5 pulse bursts, 5 Hz interburst frequency). Since [OBAA] that produces half-

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maximal inhibition of purified phospholipase A_2 (IC_{50}) is ~0.07 μM (Köhler et al., 1992), we employed a 70-fold higher concentration to achieve maximal phospholipase A_2 inhibition.

Fig. 1A compares two adult hippocampal slices. 5 μM OBAA was bath applied (hatched box) to one slice (▽), while a second, control slice (●) did not see drug. After 80 min in OBAA, both slices were given identical LFS of Schaffer collateral axons (solid bar, 1 Hz), which evoked long-term depression in both the OBAA-treated (−40%) and control (−30%) slices. Stable long-term depression lasted 60 min, after which HFS was given to both slices. In the control, stable long-term potentiation was induced (+55% of baseline). In contrast, potentiation in the OBAA-treated slice rapidly decayed to baseline within 30–40 min, in agreement with Massicotte et al. (1990).

Fig. 1B plots the mean ± S.E.M. e.p.s.p. slopes from 6 adult hippocampal slices where OBAA (5 μM) was present in the bath throughout the period of the graph. LFS evoked marked long-term depression not significantly different from long-term depression in untreated controls ($P > 0.20$, Student's t-test). In contrast, the inhibition of phospholipase A_2 did block the induction of long-term potentiation by HFS in these slices (Fig. 1A).

In light of reported opposite effects of phospholipase A_2 on AMPA binding in adult and neonatal rat brain (Baudry et al., 1991), and that both NMDA and non-NMDA forms of long-term depression are greatest in immature rats (Velíšek et al., 1993), it seemed possible that immature rats might behave differently. Therefore, I repeated these experiments on slices from 14-day-old rats. As in adults, 5 μM OBAA pretreatment ($n = 5$) did not significantly reduce the magnitude of long-term depression, while long-term potentiation was markedly impaired (data not shown).

In conclusion, a selective phospholipase A_2 inhibitor does not impair the induction of long-term depression of synaptic transmission, casting doubt on the hypothesis that activation of phospholipase A_2 is necessary for long-term depression. Doubt is strengthened by the simultaneous observation that OBAA did block induction of long-term potentiation, confirming a previous report (Massicotte et al., 1990). A recent study suggests long-term depression may require phosphatases (Mulkey et al., 1993), perhaps to dephosphorylate a site that is phosphorylated during long-term potentiation. However, my studies indicate that at least one catalytic target of long-term potentiation-associated modification by phospholipase A_2 is *not* shared by long-term depression.

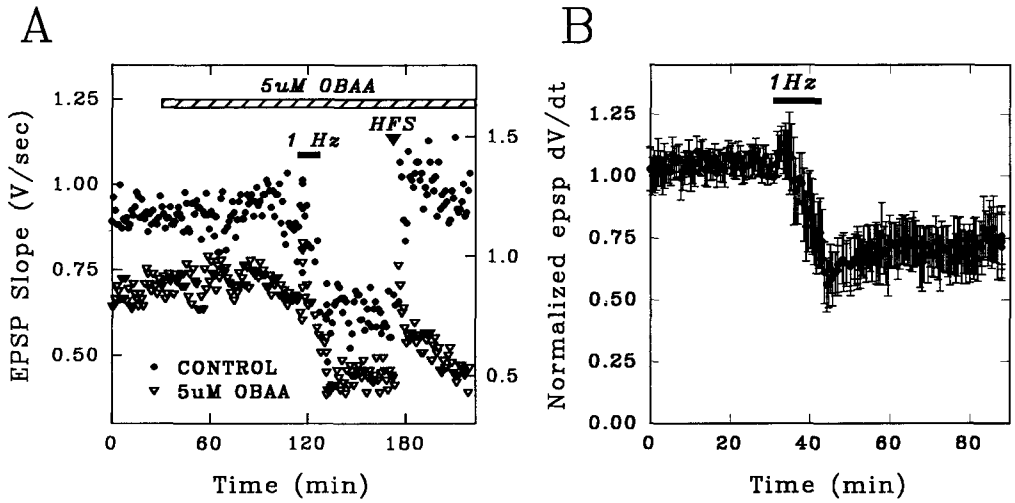

Fig. 1. Long-term depression of synaptic transmission by low-frequency stimulation is not blocked by the selective phospholipase A_2 inhibitor 3-(4-octadecyl)-benzoylacrylic acid (OBAA). A: Comparison of the effects of low-frequency Schaffer collateral stimulation (LFS; 1 Hz/15 min) on an untreated hippocampal slice (control, ●), versus a second slice (▽) pretreated with 5 μM OBAA for 80 min prior to stimulation (hatched box). E.p.s.p. slope (V/s; y-axes: left = control, right = OBAA) is plotted versus time, and both slices were taken from the same rat and recorded from in parallel in separate chambers. LFS elicited pronounced long-term depression of synaptic transmission in both control and OBAA-treated slices. Thereafter, high-frequency stimulation (HFS; 4 trains of 100 Hz theta bursts, see text) evoked persistent long-term potentiation in the control slice (+50%), whereas long-term potentiation was blocked in the OBAA-treated slice. B: Summary of 6 slices which were pretreated with 5 μM OBAA for at least 1 h prior to LFS. OBAA is present in the bathing medium prior to and throughout the time period graphed. Long-term depression was not significantly impaired by inhibition of phospholipase A_2 (Student's t-test, $P > 0.20$ compared to untreated control long-term depression). Each point is the mean ± S.E.M. of all e.p.s.p. slopes after each slope was normalized to 1.0 compared to baseline prestimulus dv/dt.

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