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Serine/Threonine Protein Phosphatases and Synaptic Inhibition Regulate the Expression of Cholinergic-Dependent Plateau Potentials

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Fraser, Douglas D., Daniel Doll, and Brian A. MacVicar. Serine/threonine protein phosphatases and synaptic inhibition regulate the expression of cholinergic-dependent plateau potentials. *J Neurophysiol* 85: 1197–1205, 2001. We previously identified cholinergic-dependent plateau potentials (PPs) in CA1 pyramidal neurons that were intrinsically generated by interplay between voltage-gated calcium entry and a Ca²⁺-activated nonselective cation conductance. In the present study, we examined both the second-messenger pathway and the role of synaptic inhibition in the expression of PPs. The stimulation of m1/m3 cholinergic receptor subtypes and G-proteins were critical for activating PPs because selective receptor antagonists (pirenzepine, hexahydro-sila-difenidol hydrochloride, 4-diphenylacetoxy-N-methylpiperidine methiodide) and intracellular guanosine-5'-O-(2-thiodiphosphate) prevented PP generation in carbachol. Intense synaptic stimulation occasionally activated PPs in the presence of oxytremorine M, a cholinergic agonist with preference for m1/m3 receptors. PPs were consistently activated by synaptic stimulation only when oxytremorine M was combined with antagonists at both GABA_A and GABA_B receptors. These latter data indicate an important role for synaptic inhibition in preventing PP generation. Both intrinsically generated and synaptically activated PPs could not be elicited following inhibition of serine/threonine protein phosphatases by calyculin A, okadaic acid, or microcystin-L, suggesting that muscarinic-induced dephosphorylation is necessary for PP generation. PP genesis was also inhibited following irreversible thiophosphorylation by intracellular perfusion with ATP- γ -S. These data indicate that the expression of cholinergic-dependent PPs requires protein phosphatase-induced dephosphorylation via G-protein-linked m1/m3 receptor(s). Moreover, synaptic inhibition via both GABA_A and GABA_B receptors normally prevents the synaptic activation of PPs. Understanding the regulation of PPs should provide clues to the role of this regenerative potential in both normal activity and pathophysiological processes such as epilepsy.

INTRODUCTION

The cholinergic system has been implicated in pathological activities such as epileptogenesis (Lothman et al. 1991; Waterlain et al. 1993). Elevations in endogenous acetylcholine are associated with seizure onset (Mizuno and Kimura 1996) and cholinergic agonists facilitate epileptogenesis in kindled animals (Buterbaugh et al. 1986). In tissue slice preparations, cholinergic receptor activation induces prolonged depolarizations (Bianchi and Wong 1994) and initiates ictal depolariza-

tions (Nagao et al. 1996; Yaari and Jensen 1989). We have previously identified a novel plateau potential (PP) in hippocampal CA1 pyramidal neurons that has characteristics similar to ictal depolarizations (Fraser and MacVicar 1996a). This regenerative PP was observed in the presence of cholinergic (Haj-Dahmane and Andrade 1998; Klink and Alonso 1997) or metabotropic glutamate (Raggenbass et al. 1997; Svirsakis and Hounsgaard 1998) agonists and requires interplay between calcium entry through high-voltage-activated (HVA) Ca²⁺ channels and a Ca²⁺-activated nonselective cation conductance (Congar et al. 1997; Crepel et al. 1994; Fraser and MacVicar 1996a). Direct enhancement of the Ca²⁺-activated nonselective cation conductance by muscarinic receptor stimulation was suggested to underlie PP expression; however, neither the muscarinic receptor subtype(s) nor the signal transduction pathway have been identified. Elucidating both the ionic mechanisms and the second-messenger pathways underlying PPs could be essential for determining the role of this PP in hippocampal epileptogenesis.

Muscarinic receptor activation modulates several voltage- and ligand-gated ion channels (Halliwell 1990; Krnjevic 1993; McCormick 1993) through a number of second-messenger pathways. These include the phosphoinositide cascade (Nicoll et al. 1990), protein kinase C (PKC) (Cantrell et al. 1996; Figenschou et al. 1996; Marsh et al. 1995; Toselli and Lux 1989; Zhang et al. 1992), Ca²⁺-calmodulin kinase II (CaMKII) (Muller et al. 1992; Pedarzani and Storm 1996), tyrosine kinase (Huang et al. 1993), and serine/threonine protein phosphatases (Herzig et al. 1995; Krause and Pedarzani 2000). Protein kinases catalyze the transfer of a phosphate from ATP to the side chain of an amino acyl residue, resulting in structural changes of the target protein (i.e., ion channels) (Hemmings et al. 1989). The degree of ion channel phosphorylation, however, depends not only on protein kinase activity, but also on protein phosphatases that catalyze dephosphorylation. Indeed, recent studies have demonstrated that the phosphorylation of ion channels results from the dynamic equilibrium between kinase and phosphatase activity (Bielefeldt and Jackson 1994; Pedarzani et al. 1998; Wang and Salter 1994; Wilson et al. 1998).

In the present study, we employed whole cell patch-clamp techniques in the hippocampal slice preparation to investigate

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the putative receptor subtype(s) and second messenger(s) underlying the expression of PPs in CA1 pyramidal neurons. We also investigated the role of synaptic inhibition on the synaptic activation of PPs. These results have been presented in abstract form (Fraser and MacVicar 1996b).

METHODS

Hippocampal slice preparation and whole cell patch-clamp recording

These techniques have been described previously (Fraser and MacVicar 1996a). Sprague-Dawley rats, *postnatal day 15–23*, were decapitated and the brain immersed in chilled artificial cerebrospinal fluid containing (ACSF; in mM): 126 NaCl, 2.5 KCl, 2 MgCl₂, 2 CaCl₂, 1.25 NaH₂PO₄, 26 NaHCO₃⁻, and 10 D-glucose; pH 7.3. The hippocampi were isolated, sectioned perpendicular to their septotemporal axis (150–400 μM), and incubated in ACSF oxygenated with 5% CO₂-95% O₂ at room temperature.

Hippocampal slices were individually transferred to a recording chamber located on an upright microscope (Standard 14; Zeiss, Thornwood, NY) and submerged in rapidly flowing (1 ml/min) oxygenated ACSF (34–35°C). Patch electrodes (5–7 MΩ) were pulled from 1.5-mm OD thin-walled glass (150F-4, World Precision Instruments) in two stages on a Narishige puller (PP-83; Tokyo, Japan) and filled with intracellular solution (in mM): 140 K-gluconate, 1.1 EGTA, 0.1 CaCl₂, 10 HEPES, 2 Mg-ATP, and 0.3 Na-GTP, pH 7.2. Intracellular Ca²⁺ concentration was calculated to be 16 nM. Voltage recordings were obtained in bridge mode (Axoclamp-2A; Axon Instruments) and were low-pass filtered (4-pole Bessel) at 10 kHz (–3 dB). Capacitance neutralization was fully maximized, and series resistance was determined via a bridge circuit potentiometer by balancing the voltage drop across the patch in response to a negative current step (–30 pA; 10 ms). Data were digitized via a TI-1 A/D interface (Axon Instruments) and analyzed using computer software (pCLAMP or Axotape). All data are presented as means ± SE. To determine statistical significance, data groups were prescreened for normality (Kolmogorov-Smirnov) and compared using a Student's paired *t*-test (SigmaStat, Jandel Scientific).

Chemicals

All salts were purchased from Fisher (Fair Lawn, NJ), Sigma (St. Louis, MO), or BDH (Toronto, ON). Carbachol (C-4832; Sigma), oxytremorine M (O-100; Sigma), atropine (A-0257; Sigma), pirenzepine (P-114; RBI; Natick, MA), 4-DAMP methiodide (D-104; RBI) and Na-orthovanadate (S-6508; Sigma) were dissolved in distilled H₂O and added to the ACSF from concentrated stocks. Also added to the ACSF, but first dissolved in DMSO, were hexahydro-sila-difenidol hydrochloride (*p*-fluoro analogue; H-127; RBI), calyculin A (C-3987;

LC Laboratories; Woburn, MA), and okadaic acid (O-2220; LC Laboratories). The final concentration of DMSO was always ≤0.1%; in control experiments, DMSO at these concentrations did not alter the cholinergic-dependent PP. Second-messenger inhibitors and analogues that were dissolved directly into the patch pipette solution included GDP-β-S (G-7637; Sigma), H-7 dihydrochloride (371955; Calbiochem; La Jolla, CA), ADP-β-S (A-8016; Sigma), 2,4-dinitrophenol (D-7004; Sigma), alkaline phosphatase (P-1153; Sigma), ATP-γ-S (A-1388; Sigma), and PP2B 476-501 (C-6481; LC Laboratories). Also added to the patch pipette solution, but first dissolved in DMSO (≤0.1%) was microcystin-LR (M-173; RBI).

During all pharmacological manipulations, control experiments were alternated with drug experiments to ensure the presence of cholinergic-dependent PPs in untreated matched slices. In addition, it was imperative that the protein phosphatase inhibitors okadaic acid and microcystin-LR were made fresh immediately before use, as these compounds demonstrated reduced inhibitory activity when taken from frozen concentrated stocks.

RESULTS

The results in this paper were obtained from 207 CA1 pyramidal neurons in the hippocampal slice preparation. The whole cell patch-clamp method was used since this technique allows for both long-duration recordings and intracellular perfusion of second-messenger inhibitors and analogues. The average access resistance obtained in control neurons was 15.3 ± 0.4 MΩ (mean ± SE, range 7–20; *n* = 110); recordings with series resistance >20 MΩ were discarded. The resting membrane potential, input resistance, membrane time constant, and action potential characteristics of the control cells were similar to our previous reports (Table 1) (Fraser and MacVicar 1996a).

Concentration dependence of the cholinergic-dependent PP

We investigated the activation of PPs in varying carbachol concentrations. In all neurons tested under control conditions, depolarizing current injection (≥0.1 nA; 0.8 s) evoked repetitive action potential firing (Fig. 1A; *n* = 225), and afterward the membrane potential immediately returned to the prestimulation baseline. We tested the actions of different concentrations of carbachol, a nonhydrolyzable cholinergic agonist, on the afterpotentials following current-evoked action potential firing. Bath application at each dose lasted for >5 min. In 0.1 μM carbachol, as in control ACSF, the membrane potential returned to prestimulation level following cessation of the current-evoked action potentials (Fig. 1B; *n* = 6). However, in 0.3 μM carbachol, a slow afterdepolarization (sADP) of 4 ± 1

TABLE 1. Membrane properties of CA1 pyramidal neurons following various pharmacological manipulations that abolished the cholinergic-dependent PP

	rp, mV	R _{IN} , MΩ	AP _{AMP} , mV	AP _{TH} , mV	AP _{DUR} , ms	τ, ms	<i>n</i>
Control	–65 ± 0.3	146 ± 4	94 ± 1	–46 ± 0.4	1.6 ± 0.02	21.7 ± 0.6	110
GDP-β-S	–58 ± 1.2	136 ± 10	101 ± 1	–40 ± 0.9	1.9 ± 0.07	16.9 ± 0.6	3
ATP-γ-S	–65 ± 0.7	116 ± 11	93 ± 1	–47 ± 2.2	2.1 ± 0.09	19.7 ± 4.5	6
cal A	–58 ± 1.5	152 ± 5	96 ± 2	–45 ± 1.8	1.9 ± 0.09	17.7 ± 1.2	9
OA	–58 ± 1.7	143 ± 12	95 ± 2	–44 ± 1.2	1.8 ± 0.07	17.7 ± 0.7	6
m-LR	–59 ± 3.5	137 ± 6	83 ± 6	–43 ± 1.7	1.7 ± 0.08	19.3 ± 1.4	6

Values are means ± SE; *n* is number of neurons. Input resistance (R_{IN}) was calculated from a steady-state potential response, free of membrane rectification, to a hyperpolarizing current step. The membrane time constant (τ) was calculated as the time necessary to reach 1 – e^{–1} (63%) of the maximum voltage deflection. The action potential threshold (AP_{TH}) was measured as the membrane potential at the base of the action potential, whereas action potential amplitude (AP_{AMP}) was measured as the voltage difference between the threshold and peak amplitude. Action potential duration (AP_{DUR}) was measured at the threshold potential. rp, resting membrane potential; cal A, calyculin A; OA, okadaic acid; m-LR, microcystin-LR.

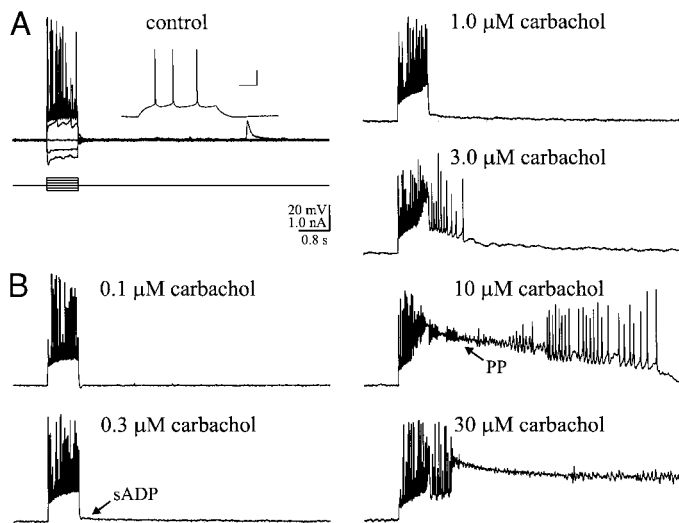


FIG. 1. Increasing concentrations of carbachol revealed a cholinergic-dependent slow afterdepolarization (sADP) and plateau potential (PP). *A*: in control artificial cerebrospinal fluid (ACSF), depolarizing current injection elicited repetitive action potential firing in a CA1 pyramidal neuron. The membrane potential immediately returned to baseline levels following cessation of the current pulse. The *inset* illustrates the firing pattern of this neuron in response to a 0.2-s depolarizing current stimulus. The resting membrane potential of this neuron was -62 mV. *B*: bath application of variable concentrations of carbachol revealed a cholinergic-dependent sADP and PP. The sADP was observed following a 5-min incubation in 0.3 μ M carbachol (arrow). The sADP elicited by burst firing increased in amplitude and duration following incubation in increasing concentrations of carbachol (1.0 ; 3.0 μ M). Application of carbachol concentrations >10 μ M revealed a cholinergic-dependent PP elicited by action potential firing (arrow).

mV and 3.5 ± 0.4 s was observed immediately following the depolarizing current stimulus (Fig. 1*B*; $n = 6/6$). A larger amplitude sADP was evoked when the carbachol concentration was increased to 1 μ M (7 ± 1 mV; 4.3 ± 0.4 s; $n = 6/6$). While action potential firing in 3 μ M carbachol elicited a larger sADP in one-half the neurons (14 ± 3 mV; 6.5 ± 0.4 s; $n = 3/6$), a regenerative PP was observed in the remaining cells ($n = 3/6$). The membrane potential and duration of the PPs were -30 ± 8 mV and 2.8 ± 0.2 s, respectively. In the three remaining neurons exhibiting only a sADP at 3 μ M, PPs were observed at a concentration of 10 μ M. The membrane potential and duration of the PPs in 10 μ M carbachol were -20 ± 5 mV and 6.2 ± 0.9 s, respectively ($n = 6/6$). At the highest concentration of carbachol tested (30 μ M), PPs were elicited in all neurons ($n = 6/6$). These data demonstrate a clear concentration dependence of the cholinergic-dependent PP. A critical level of receptor stimulation must be obtained between 3 and 10 μ M carbachol before a PP can be generated consistently. We therefore used concentrations of 20 μ M to elicit PPs so that we could examine drug effects.

Effects of muscarinic receptor antagonists

As reported previously, the PPs were abolished following co-application of 1 μ M atropine with 20 μ M carbachol; although a small sADP was still elicited (2 ± 1 mV; 2.9 ± 0.5 s; $n = 5/5$) (Fraser and MacVicar 1996a). Since atropine is a nonselective antagonist of all muscarinic receptors, we tested the ability of relatively selective muscarinic receptor antagonists to suppress the PP elicited in the presence of 20 μ M carbachol (Fig. 2). Coapplication of either 1 μ M pirenzepine

($n = 3$), an antagonist with greater affinity for m1 over m3 receptors (Dorje et al. 1991), or 1 μ M 4-diphenylacetoxy-*N*-methylpiperidine methiodide ($n = 3$; 4-DAMP), an antagonist with equal affinity to both m1 and m3 receptors (Michel et al. 1989; Thomas et al. 1992), abolished cholinergic-dependent PPs. Coapplication of 1 μ M hexahydro-sila-difenidol hydrochloride (HHSiD; *p*-fluoro analog), an antagonist with greater affinity for m3 over m1 receptors (Lambrecht et al. 1989), also inhibited cholinergic-dependent PPs ($n = 3/4$; data not shown). These data implicate m1 and/or m3 receptors in cholinergic-dependent PP genesis.

G-protein involvement in the cholinergic-dependent PP

Muscarinic receptors are coupled to effector systems via G-proteins (Brann et al. 1993), and some studies have shown that G-proteins are involved in activation of hippocampal cation currents (Crepel et al. 1994). However, other recent studies have excluded G-protein involvement in the muscarinic activation of a nonselective cation conductance in cultured neurons (Brown et al. 1993; Guerineau et al. 1995). To determine whether G-proteins are involved in the generation of the cholinergic-dependent PP, we substituted 2 mM guanosine-5'-*O*-(2-thiodiphosphate) (GDP- β -S) for GTP in the patch pipette solution. GDP- β -S is a hydrolysis-resistant guanine nucleotide that inhibits receptor-induced activation of G-proteins (Andrade 1994). Intracellular dialysis of hippocampal CA1 pyramidal neurons with this compound abolished both the initial membrane depolarization elicited by application of 20 μ M carbachol (data not shown; $n = 3/3$) and the cholinergic-dependent PP ($n = 3/3$, Fig. 3). These data indicate that G-proteins are a necessary component of the cholinergic signal transduction pathway both in mediating the initial depolarization and in the expression of the PP.

Role of dephosphorylation and protein phosphorylation

The regulation of ion channels by phosphatases has recently been demonstrated (Bielefeldt and Jackson 1994; Wang and Salter 1994; White et al. 1991), suggesting that dephosphorylation may be important in the modulation of neuronal responses. To test whether dephosphorylation plays a role in PP genesis, ATP in the pipette solution was replaced with 5 mM adenosine-5'-*O*-(3-thiotriphosphate) (ATP- γ -S). We assumed that there was some basal degree of protein kinase activity, and therefore ATP- γ -S was included in the patch pipette solution to

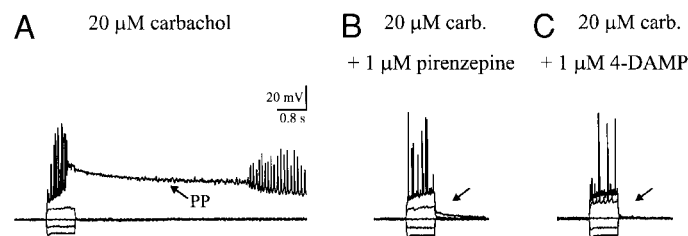


FIG. 2. The cholinergic-dependent PP was abolished by m1/m3 receptor antagonists. *A*: a cholinergic-dependent PP was elicited by burst firing in a CA1 pyramidal neuron. *B*: in another neuron, coapplication of 1 μ M pirenzepine with 20 μ M carbachol (carb.) abolished the cholinergic-dependent PP. A small sADP, however, was still evoked in the presence of pirenzepine (arrow). *C*: coapplication of 1 μ M 4-diphenylacetoxy-*N*-methylpiperidine methiodide (4-DAMP) with 20 μ M carbachol (carb.) abolished both the sADP and the PP in the illustrated neuron (arrow).

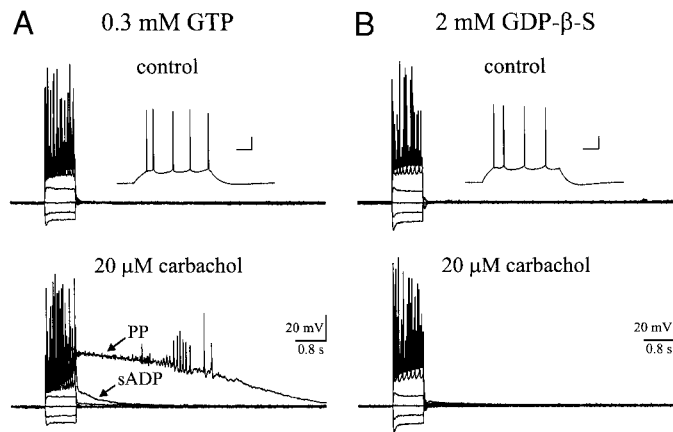


FIG. 3. The cholinergic-dependent PP was abolished by inclusion of GDP- β -S in the patch pipette solution. *A*: in the absence of carbachol, depolarizing current injection elicits action potential firing in a CA1 pyramidal neuron. The membrane potential returned to baseline following cessation of current injection. The pipette solution contained 0.3 mM GTP. The *inset* illustrates the firing pattern of this neuron in response to a 0.2-s depolarizing current stimulus. In contrast to control, action potential firing in the presence of 20 μ M carbachol elicited a sADP and PP following cessation of the current stimuli. The resting membrane potential of this neuron in control and carbachol was -66 and -61 mV, respectively. *B*: in this CA1 pyramidal neuron, 2 mM GDP- β -S was substituted for GTP in the patch-pipette solution. As before, depolarizing current injection elicited action potential firing in control ACSF. The membrane potential returned to baseline following cessation of the current stimuli. The *inset* illustrates the firing pattern of this neuron in response to a 0.2-s depolarizing current stimulus. Application of 20 μ M carbachol failed to depolarize this neuron from a membrane potential held at -60 mV (-0.04 nA) and the cholinergic-dependent PP could not be evoked by evoked action potential firing.

irreversibly thiophosphorylate substrate proteins. Neurons were internally perfused with this compound for a minimum of 20 min prior to data collection. Following this intracellular perfusion, the initial depolarization induced by carbachol activation was significantly reduced to $48 \pm 14\%$ of control ($P \leq 0.038$, $n = 6$). Furthermore, expression of the cholinergic-dependent PP was abolished (Fig. 4, *A* and *B*; $n = 6/6$). These data indicate that dephosphorylation was necessary for expression of cholinergic-dependent PPs.

We tested whether inhibitors of protein phosphatases could block the expression of cholinergic PPs because the above data implicated that dephosphorylation was necessary. Several approaches were used to test for the involvement of serine/threonine protein phosphatases. First, hippocampal slices (150–450 μ m) were incubated in either calyculin A (cal A) or okadaic acid (OA) at concentrations of 1–2 μ M for >3 h. This experimental approach has been used successfully in previous studies, indicating that these inhibitors permeate neurons in tissue slices (Mulkey et al. 1993; Muller et al. 1992). Second, the cell-impermeable protein phosphatase inhibitor microcystin-LR (m-LR), was applied internally via the patch pipette at a concentration of 10 μ M (Mulkey et al. 1993). In this latter experiment, data collection began only after 20 min of internal perfusion. The blockade of serine/threonine protein phosphatases, by either calyculin A, okadaic acid, or microcystin-LR, resulted in a depolarized resting membrane potential (Table 1) and a reduction in the depolarization induced by 20 μ M carbachol (cal A, decreased to $33 \pm 21\%$ of control, $P < 0.001$, $n = 7$; OA, decreased to $44 \pm 9\%$ of control, $P \leq 0.003$, $n = 7$; m-LR, decreased to $47 \pm 25\%$ of control, $P \leq 0.008$, $n =$

6). Finally, the cholinergic-dependent PP could not be evoked in most neurons recorded from slices incubated in either cal A (Fig. 4*C*; $n = 12/14$ slices) or OA (Fig. 4*D*; $n = 6/7$ slices). The cholinergic-dependent PP was also abolished in 67% of neurons intracellularly loaded with m-LR (Fig. 4*E*; $n = 4/6$).

To examine the involvement of other protein phosphatase subtypes, we also tested specific inhibitors of either protein phosphatase 2B (calcineurin) or tyrosine phosphatase. To inhibit calcineurin, the selective peptide inhibitor 476-501 (100 μ M) (Hendey et al. 1992) was internally perfused via the patch-clamp electrode. At 100 μ M, peptide 476-501 does not

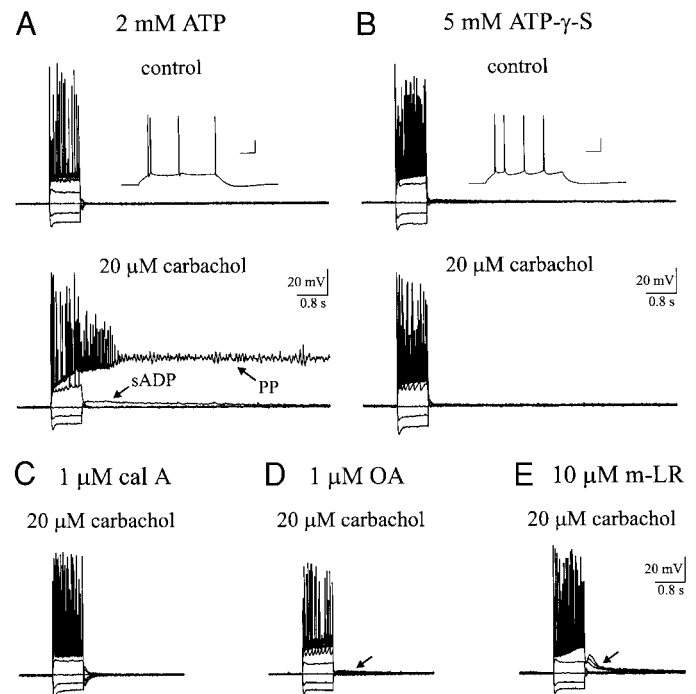


FIG. 4. Expression of the cholinergic-dependent PP required serine/threonine protein phosphatase-induced dephosphorylation. *A*: in control ACSF, depolarizing current injection elicited action potential firing in a CA1 pyramidal neuron. The membrane potential returned to baseline following cessation of current injection. The pipette solution contained 2 mM ATP. The *inset* illustrates the firing pattern of this neuron in response to a 0.2-s depolarizing current stimulus. In the presence of 20 μ M carbachol, action potential firing elicited a sADP and PP following cessation of the current stimuli. The resting membrane potential of this neuron in control and carbachol was -64 and -58 mV, respectively. *B*: action potential firing elicited by depolarizing current injection in a CA1 pyramidal neuron. The membrane potential returned to baseline following cessation of the stimuli. In this cell, the recording pipette solution contained 5 mM ATP- γ -S. Neurons were dialyzed with this compound for an obligatory 20-min period before data collection. Under these conditions, the cholinergic-dependent PP could not be elicited by burst firing. The resting membrane potential of this neuron in control and carbachol was -64 and -62 mV, respectively. *C*: cholinergic-dependent PPs could not be elicited in a hippocampal slice incubated in 1 μ M of the cell-permeable protein phosphatase inhibitors calyculin A (cal A). The membrane potential of this neuron was held at -62 mV (-0.09 nA) and depolarized in the presence of carbachol to -60 mV. *D*: in another hippocampal neuron, action potential firing failed to elicit a cholinergic-dependent PP following incubation in 1 μ M okadaic acid (OA) for >3 h prior to data collection. The membrane potential of this neuron was held at -60 mV (-0.1 nA) and depolarized in the presence of carbachol to -58 mV. *E*: cholinergic-dependent PPs could not be generated in neurons dialyzed with a recording pipette solution containing 10 μ M microcystin-LR (m-LR). Neurons were dialyzed with this compound for an obligatory 20-min period before data collection. The membrane potential of this neuron was held at -60 mV (-0.17 nA) and depolarized in the presence of carbachol to -57 mV.

inhibit serine/threonine protein phosphatases (Hendey et al. 1992). Dialysis of neurons for 20 min with this peptide inhibitor did not significantly depress the initial cholinergic-induced depolarization (decreased to $90 \pm 24\%$ of control, $n = 4$) and was also ineffective in inhibiting the cholinergic-dependent PP (data not shown, $n = 4/4$). To test for the involvement of tyrosine phosphatase, hippocampal slices were first incubated in $100 \mu\text{M}$ Na-orthovanadate for >2 h, and this same concentration of inhibitor was included in the patch pipette solution. At $100 \mu\text{M}$, Na-orthovanadate has virtually no dephosphorylating effect on serine-threonine phosphoproteins (Swarup et al. 1982). This inhibitor neither affected the resting membrane potential nor significantly depressed the initial cholinergic-induced depolarization (decreased to $94 \pm 16\%$ of control, $n = 4$). In addition, the cholinergic-dependent PP was consistently elicited (data not shown, $n = 4/4$), suggesting that tyrosine phosphatase is not a component of this signal transduction cascade. Hence these data exclude the involvement of calcineurin or tyrosine phosphatase in expression of the cholinergic-dependent PP.

Lack of effect of protein kinase inhibition

Our experiments with ATP- γ -S and serine/threonine protein phosphatase inhibitors indicated that dephosphorylation was critical for the expression of cholinergic-dependent PPs. Protein kinases are reported to activate protein phosphatases directly in some signal transduction pathways (Surmeier et al. 1995; Wilson and Kaczmarek 1993). Therefore we tested whether internal perfusion of $300 \mu\text{M}$ H-7, a potent, but nonselective kinase inhibitor (Hidaka et al. 1991; Ruegg and Burgess 1989), could abolish PPs elicited in $20 \mu\text{M}$ carbachol (Fraser et al. 1993; Malenka et al. 1989; Malinow et al. 1989; Zhang et al. 1992). The initial cholinergic-induced depolarization was significantly reduced by H-7 in the patch pipette solution (decreased to $64 \pm 8\%$ of control; $P \leq 0.032$, $n = 4$). In contrast, H-7 did not block the expression of cholinergic-dependent PPs elicited by action potential firing (Fig. 5A; $n = 4/4$). These data imply that, although protein kinase(s) mediated some of the initial cholinergic-induced depolarization, there is no apparent involvement of protein kinases in expression of the cholinergic-dependent PP.

We next determined the potential role for phosphorylation in the expression of PPs elicited in $20 \mu\text{M}$ carbachol by internally perfusing a phosphorylation-inhibiting cocktail (PIC) containing 0 Mg-ATP , $5 \text{ mM ADP-}\beta\text{-S}$, $50 \mu\text{M}$ dinitrophenol, and 1 mg/ml alkaline phosphatase (Chen and Smith 1992; Chen et al. 1990; Hescheler et al. 1987; Shuba et al. 1990). The resting membrane potentials of neurons loaded with PIC were significantly more depolarized than control neurons (PIC $-59 \pm 2 \text{ mV}$, $n = 9$; see Table 1 for control values), and the initial cholinergic-induced depolarization was significantly depressed in neurons containing PIC (decreased to $40 \pm 14\%$ of control, $P \leq 0.017$, $n = 4$). In contrast, the cholinergic-dependent PP was consistently elicited by evoked action potential firing in the presence of $20 \mu\text{M}$ carbachol (Fig. 5B; $n = 4/4$). This experiment

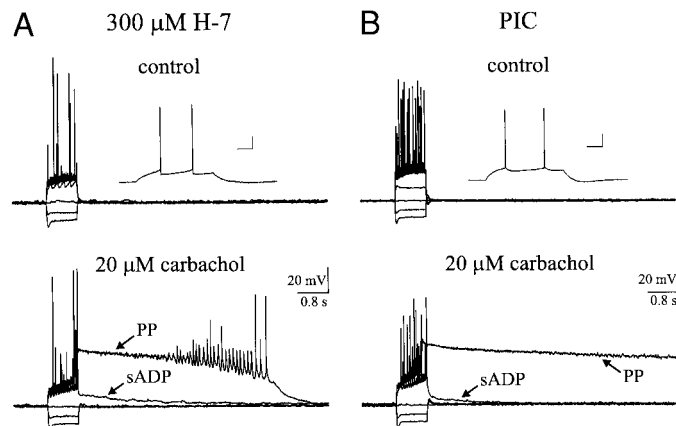


FIG. 5. Cholinergic-dependent PPs were unaffected by intracellular perfusion of either a nonselective protein kinase inhibitor or a phosphorylation-inhibiting cocktail. *A*: action potential firing elicited by depolarizing current injection in a CA1 pyramidal neuron. The membrane potential returned to baseline following cessation of the stimuli. In this cell, the recording pipette solution contained $300 \mu\text{M}$ of the nonselective protein kinase inhibitor H-7. Despite intracellular dialysis with this compound, a PP was still evoked in the presence of $20 \mu\text{M}$ carbachol. The resting membrane potential of this neuron in control and carbachol was -63 and -60 mV , respectively. *B*: action potential firing elicited by depolarizing current injection in a CA1 pyramidal neuron. The membrane potential returned to baseline following cessation of the stimuli. In this cell, the recording pipette solution contained a phosphorylation-inhibiting cocktail (PIC; see RESULTS for contents). Despite intracellular dialysis with this compound, a PP was still evoked in the presence of $20 \mu\text{M}$ carbachol. The membrane potential of this neuron was held at -65 mV (-120 pA) and depolarized in the presence of carbachol to -63 mV .

further indicates that phosphorylation is unnecessary for the expression of cholinergic-dependent PPs.

Synaptic activation of PPs and GABA antagonists

The activation of PPs by current-evoked action potentials is an all-or-none event (Fraser and MacVicar 1996a). We investigated whether synaptic activation could also evoke all-or-none PPs in CA1 pyramidal neurons by stimulating the Schaffer collaterals while bath applying a cholinergic agonist (carbachol or oxotremorine M). Oxotremorine M was sometimes used because it has slight preference for m1/m3 as compared with m2 cholinergic receptors (Brann et al. 1993), and we showed above that m1/m3 receptors mediate PP genesis. In the presence of either carbachol ($20 \mu\text{M}$) or oxotremorine M ($15 \mu\text{M}$) alone, synaptic stimulation rarely activated PPs ($n = 2/6$). In contrast, intracellular current injection always evoked PPs in these same neurons ($n = 6/6$). Synaptic stimulation did not evoke PPs even though the synaptic-induced depolarization was equivalent to the depolarization elicited by intracellular current injection that resulted in PP genesis (Fig. 6A). As inhibitory circuits are also activated by synaptic stimulation, we perfused GABAergic antagonists to determine whether synaptic stimulation would elicit PPs when inhibition was depressed. Antagonists of GABA_A receptors ($30 \mu\text{M}$ bicuculline) and GABA_B receptors ($50 \mu\text{M}$ CGP 35348) alone did not unmask synaptically stimulated PPs ($n = 0/6$; Fig. 6B). Coapplication of oxotremorine M with either the GABA_A antagonist bicuculline ($30 \mu\text{M}$; $n = 5$), or with the GABA_B receptor antagonist CGP 35348 ($50 \mu\text{M}$; $n = 6$) also did not result in PP genesis with synaptic stimulation (data not shown). However, PPs were consistently evoked under these conditions

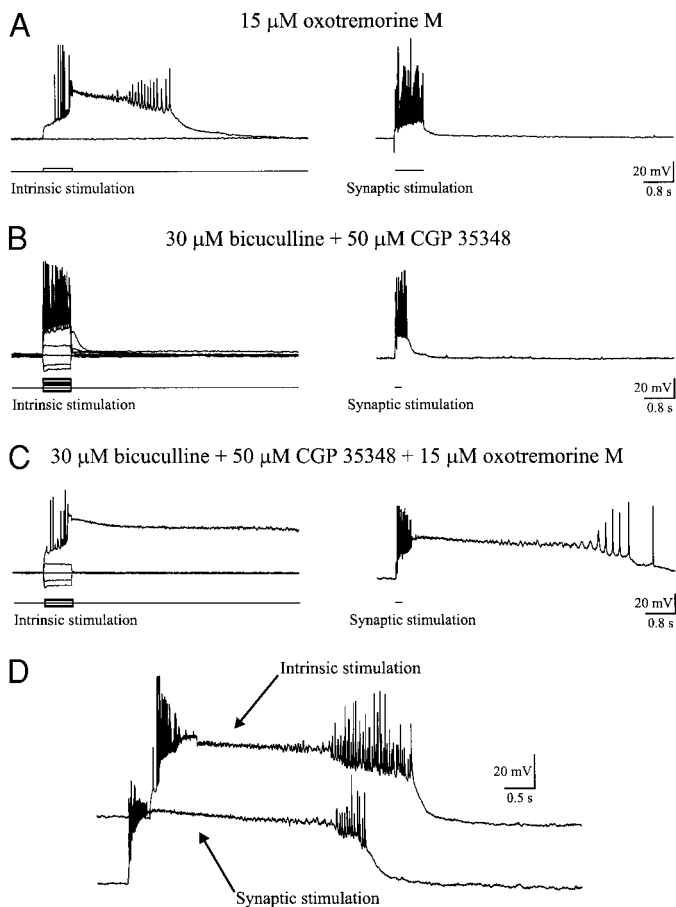


FIG. 6. Synaptic stimulation evoked PPs only when muscarinic agonists were coapplied with GABA receptor antagonists. *A*: PPs were consistently elicited by intracellular current injection in the presence of the cholinergic agonist oxotremorine M (15 μ M). Under these same conditions, stimulation of the Schaffer collaterals failed to trigger PP genesis. *B*: application of both GABA_A and GABA_B receptor antagonists in the absence of cholinergic stimulation failed to unmask PP genesis by either intrinsic or synaptic stimulation. *C*: both intrinsic and synaptic stimulation consistently elicited PPs when the application of both GABA_A and GABA_B receptor antagonists was combined with the cholinergic agonist oxotremorine M. *D*: both an intrinsically activated PP and a synaptically activated PP are illustrated to demonstrate the similarities in waveform, despite the different methods of stimulation.

by intracellular current injection ($n = 8/11$; oxotremorine M, 15 μ M; data not shown). In contrast, when both GABA_A and GABA_B antagonists (bicuculline 30 μ M and CGP 35348 50 μ M) were bath applied in conjunction with oxotremorine M, synaptic stimulation consistently evoked PPs ($n = 21/24$; Fig. 6C). These results indicate that depression of both GABA_A- and GABA_B-mediated synaptic inhibition and activation of muscarinic receptors was necessary for the expression of synaptically driven PPs. In any one cell, the waveform of the synaptically evoked PP was remarkably similar to the PP evoked intrinsically by current injection (Fig. 6D). Similar to our previous report on intrinsically generated PPs (Fraser and MacVicar 1996a), PPs were not evoked by synaptic stimulation in oxotremorine M, bicuculline, and CGP 34358 when 10 mM bis-(*o*-aminophenoxy)-*N,N,N',N'*-tetraacetic acid was included in the patch pipette to chelate intracellular calcium ($n = 0/4$; data not shown). Therefore synaptic stimulation appears to evoke an intrinsically generated all-or-none PP when musca-

rinic stimulation is combined with depression of synaptic inhibition.

DISCUSSION

We have previously identified a novel cholinergic-dependent PP in CA1 pyramidal neurons, which relies on the interaction between HVA Ca²⁺ channels and the Ca²⁺-activated nonselective cation conductance (Fraser and MacVicar 1996a). This PP is observed in the presence of cholinergic (or metabotropic glutamate) agonists and is similar to ictal depolarizations observed during cholinergic-induced seizures (Nagao et al. 1996; Yaari and Jensen 1989). In this paper, we have elucidated the muscarinic receptors and various components of the signal transduction pathway underlying the expression of cholinergic-dependent PPs. Our evidence suggests that m1/m3 receptors are coupled to serine/threonine protein phosphatases, either directly or indirectly via G-proteins. The expression of cholinergic-dependent PPs therefore requires phosphatase-induced dephosphorylation. We have also demonstrated that, similar to the generation of ictal depolarizations, the synaptic activation of the PP is facilitated by depression of inhibition.

The expression of the cholinergic-dependent PP appears to be mediated by m1 and/or m3 receptors. The presence of both m1/m3 receptor subtypes in hippocampal tissue is well-documented by anatomical studies (Vilaro et al. 1993), and m1/m3 receptor stimulation is implicated in the modulation of cellular excitability (Auerbach and Segal 1996; Cox et al. 1994). The binding of m1/m3 receptor antagonists, [³H]pirenzepine and [³H]4-DAMP, is maximal in the rat hippocampus relative to other structures (Aubert et al. 1996), and these antagonists inhibit phosphoinositide metabolism, suggesting that stimulation of m1/m3 receptors liberate this second messenger (Candura et al. 1995). The phosphoinositide signal transduction cascade activated by m1/m3 receptors is mediated by a G-protein (G_{q/11}) (Vilaro et al. 1993), and in agreement with this we have found that the effects of cholinergic stimulation were occluded by GDP- β -S. Indeed, several studies have demonstrated that muscarinic or metabotropic glutamate receptor stimulation activates phospholipase C via G-proteins, resulting in IP₃ production and elevated intracellular Ca²⁺ (Kostyuk and Verkhratsky 1994). Intracellular Ca²⁺ elevated by these neurotransmitters stimulates the Ca²⁺-activated nonselective cation conductance, thereby depolarizing the membrane potential (Congar et al. 1997; Crepel et al. 1994).

We have demonstrated critical roles for protein phosphatases and dephosphorylation in the expression of cholinergic-dependent PPs. The involvement of dephosphorylation was first tested using intracellular perfusion of ATP- γ -S, which irreversibly thiophosphorylates substrate proteins. Intracellular dialysis of this compound depressed the initial cholinergic-induced depolarization and abolished the PPs elicited in carbachol. As dephosphorylation is mediated by protein phosphatases, we then investigated whether inhibitors of protein phosphatases prevented PP genesis. Both membrane-permeable (calyculin A and okadaic acid) and -impermeable (m-LR) inhibitors of serine/threonine protein phosphatases abolished the cholinergic-dependent PP. The effectiveness of these inhibitors in our study is similar to a previous study of protein phosphatases in hippocampal slices (Mulkey et al. 1993). Inhibitors of calcineurin (peptide 476-501) or tyrosine phosphatase

tase (Na^+ -orthovanadate) did not, however, affect PP genesis. The expression of cholinergic-dependent PPs therefore required the activation of serine/threonine protein phosphatases, either directly or indirectly via G-proteins. A direct activation of protein phosphatases by G-proteins has been suggested previously (Bielefeldt and Jackson 1994). Recently, muscarinic modulation of calcium-activated potassium conductances has also been shown to rely on protein phosphatase activation (Krause and Pedarzani 2000).

Protein phosphatases are directly activated by protein kinases in some systems (Surmeier et al. 1995; Wilson and Kaczmarek 1993), and a variety of protein kinases are activated by muscarinic stimulation (PKC, Figenschou et al. 1996; Marsh et al. 1995; Zhang et al. 1992; CaMKII, Muller et al. 1992; Pedarzani and Storm 1996; tyrosine kinase, Huang et al. 1993). Although the nonselective protein kinase inhibitor H-7 depressed the initial cholinergic-induced depolarization in the present study, no inhibition of the cholinergic-dependent PP was observed. This finding suggested that PP genesis may be independent of protein kinase activity. To further test this possibility, we internally perfused neurons with PIC, a phosphorylation-inhibiting cocktail (Chen and Smith 1992; Chen et al. 1990; Hescheler et al. 1987; Shuba et al. 1990). Similar to the H-7 experiments, the initial cholinergic-induced depolarization was depressed by PIC. The cholinergic-dependent PP was still generated, however, again demonstrating that phosphorylation is not involved in PP genesis.

What is the target for phosphatase-induced dephosphorylation? We have previously demonstrated that the PP relies on interactions between calcium entry through HVA Ca^{2+} channels and the Ca^{2+} -activated cation conductance (Fraser and MacVicar 1996a). We further postulated that this latter conductance is directly enhanced by cholinergic stimulation. Based on the data presented here, we postulate that the target for dephosphorylation is a serine/threonine site on the Ca^{2+} -activated nonselective cation channel. This theory is consistent with previous reports demonstrating that the open channel time and Ca^{2+} sensitivity of nonselective cation channels are greatly reduced by PKA-induced phosphorylation in both invertebrate (Partridge et al. 1990) and vertebrate neurons (Razani-Boroujerdi and Partridge 1993). Elevations in the activity of either cAMP or PKA also depress the Ca^{2+} -activated nonselective cation channel recorded from cochlea (Van den Abbeele et al. 1996) or insulinoma (Reale et al. 1995) cells. Consistent with these findings, we have shown that the PP could not be generated in the presence ATP- γ -S or inhibitors of serine/threonine phosphatases, indicating the importance of dephosphorylation in PP genesis. Interestingly, the conditions that reduce PP generation also enhance activity of HVA Ca^{2+} channels. For example, L-type Ca^{2+} currents in hippocampal neurons were enhanced by elevations in intracellular PKA activity (Chetkovich et al. 1991; Hell et al. 1995) or by protein phosphatase inhibitors (Mironov and Lux 1991). Given that PPs rely on both HVA Ca^{2+} channels and the Ca^{2+} -activated nonselective cation conductance, and that Ca^{2+} influx was probably augmented by protein phosphatase inhibition, it is possible that the Ca^{2+} -activated nonselective cation conductance was depressed to a much greater extent than is evident from our observations.

We have previously suggested that the PP is an excellent candidate for an intrinsic mechanism underlying ictal depolar-

izations observed during both experimental (Nagao et al. 1996; Yaari and Jensen 1989) and clinical (Lothman et al. 1991) epileptogenesis. We have found that synaptic stimulation can elicit PPs, but only when synaptic inhibition is depressed by GABA receptor antagonists. Depression of synaptic inhibition is also an important trigger for eliciting seizures in vivo (Lothman et al. 1991). Our results support the hypothesis that the ictal depolarization of epilepsy is an all-or-none PP that can be triggered by spike activity or synaptic inputs (Fraser and MacVicar 1996a). In addition, it has recently been demonstrated that a clinically relevant anticonvulsant (10–100 μM topiramate) depresses the cholinergic-dependent PP in subicular neurons (Palmieri et al. 2000). During interictal bursting, elevated levels of ACh and glutamate could stimulate muscarinic and metabotropic receptors, respectively. Activation of the protein phosphatase pathway via these receptors, in conjunction with increased intracellular calcium, may lead to PP genesis as described in this study. Recurrent seizures can, as a consequence, induce profound hypoglycemia (Wasterlain et al. 1993) and precipitous decreases in ATP and phosphocreatinine levels (DeFrance and McCandless 1991; Fujikawa et al. 1988). These conditions would favor dephosphorylation and possibly exacerbate existing seizure activity. As the PP represents a feed-forward regenerative potential that results in prolonged depolarization and maintained Ca^{2+} influx, these mechanisms may also represent a crucial component of excitotoxicity (Chen et al. 1997).

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REFERENCES

- ANDRADE R. Infusion of guanine nucleotides through recording electrodes for studies on G-protein regulation of ion currents and channels. *Methods Enzymol* 238: 348–356, 1994.
- AUBERT I, CECYRE D, GAUTHIER S, AND QUIRION R. Comparative ontogenic profile of cholinergic markers, including nicotinic and muscarinic receptors, in the rat brain. *J Comp Neurol* 369: 31–55, 1996.
- AUERBACH JM AND SEGAL M. Muscarinic receptors mediating depression and long-term potentiation in rat hippocampus. *J Physiol (Lond)* 492: 479–493, 1996.
- BIANCHI R AND WONG RK. Carbachol-induced synchronized rhythmic bursts in CA3 neurons of guinea pig hippocampus in vitro. *J Neurophysiol* 72: 131–138, 1994.
- BIELEFELDT K AND JACKSON MB. Phosphorylation and dephosphorylation modulate a Ca^{2+} -activated K^+ channel in rat peptidergic nerve terminals. *J Physiol (Lond)* 475: 241–254, 1994.
- BRANN MR, ELLIS J, JORGENSEN H, HILL-EUBANKS D, AND JONES SV. Muscarinic acetylcholine receptor subtypes: localization and structure/function. *Prog Brain Res* 98: 121–127, 1993.
- BROWN LD, KIM KM, NAKAJIMA Y, AND NAKAJIMA S. The role of G protein in muscarinic depolarization near resting potential in cultured hippocampal neurons. *Brain Res* 612: 200–209, 1993.
- BUTERBAUGH GG, MICHELSON HB, AND KEYSER DO. Status epilepticus facilitated by pilocarpine in amygdala-kindled rats. *Exp Neurol* 94: 91–102, 1986.
- CANDURA SM, TONINI M, BAIARDI P, MANZO L, AND COSTA LG. Heterogeneity of cholinergic muscarinic receptors coupled to phosphoinositide metabolism in immature rat brain. *Brain Res Dev Brain Res* 86: 134–142, 1995.

- CANTRELL AR, MA JY, SCHEUER T, AND CATTERALL WA. Muscarinic modulation of sodium current by activation of protein kinase C in rat hippocampal neurons. *Neuron* 16: 1019–1026, 1996.
- CHEN H AND SMITH PA. M-currents in frog sympathetic ganglion cells: manipulation of membrane phosphorylation. *Br J Pharmacol* 105: 329–334, 1992.
- CHEN QX, PERKINS KL, CHOI DW, AND WONG RKS. Secondary activation of a cation conductance is responsible for NMDA toxicity in acutely isolated hippocampal neurons. *J Neurosci* 17: 4032–4036, 1997.
- CHEN QX, STELZER A, KAY AR, AND WONG RK. GABAA receptor function is regulated by phosphorylation in acutely dissociated guinea-pig hippocampal neurones. *J Physiol (Lond)* 420: 207–221, 1990.
- CHETKOVICH DM, GRAY R, JOHNSTON D, AND SWEATT JD. N-methyl-D-aspartate receptor activation increases cAMP levels and voltage-gated Ca^{2+} channel activity in area CA1 of hippocampus. *Proc Natl Acad Sci USA* 88: 6467–6471, 1991.
- CONGAR P, LEINEKUGEL X, BEN-ARI Y, AND CREPEL V. A long-lasting calcium-activated nonselective cationic current is generated by synaptic stimulation or exogenous activation of group I metabotropic glutamate receptors in CA1 pyramidal neurons. *J Neurosci* 17: 5366–5379, 1997.
- COX CL, METHERATE R, AND ASHE JH. Modulation of cellular excitability in neocortex: muscarinic receptor and second messenger-mediated actions of acetylcholine. *Synapse* 16: 123–136, 1994.
- CREPEL V, ANIKSZTEIN L, BEN-ARI Y, AND HAMMOND C. Glutamate metabotropic receptors increase a Ca^{2+} -activated nonselective cationic current in CA1 hippocampal neurons. *J Neurophysiol* 72: 1561–1569, 1994.
- DEFRANCE JF AND MCCANDLESS DW. Energy metabolism in rat hippocampus during and following seizure activity. *Metab Brain Dis* 6: 83–91, 1991.
- DORJE F, WESS J, LAMBRECHT G, TACKE R, MUTSCHLER E, AND BRANN MR. Antagonist binding profiles of five cloned human muscarinic receptor subtypes. *J Pharmacol Exp Ther* 256: 727–733, 1991.
- FIGENSCOU A, HU GY, AND STORM JF. Cholinergic modulation of the action potential in rat hippocampal neurons. *Eur J Neurosci* 8: 211–219, 1996.
- FRASER DD, HOEHN K, WEISS S, AND MACVICAR BA. Arachidonic acid inhibits sodium currents and synaptic transmission in cultured striatal neurons. *Neuron* 11: 633–644, 1993.
- FRASER DD AND MACVICAR BA. Cholinergic-dependent plateau potential in hippocampal CA1 pyramidal neurons. *J Neurosci* 16: 4113–4128, 1996a.
- FRASER DD AND MACVICAR BA. Expression of cholinergic-dependent plateau potentials requires phosphatase-induced dephosphorylation. *Soc Neurosci Abstr* 22: 313.7, 1996b.
- FUJIKAWA DG, VANNUCCI RC, DWYER BE, AND WASTERLAIN CG. Generalized seizures deplete brain energy reserves in normoxemic newborn monkeys. *Brain Res* 454: 51–59, 1988.
- GUERINEAU NC, BOSSU JL, GAHWILER BH, AND GERBER U. Activation of a nonselective cationic conductance by metabotropic glutamatergic and muscarinic agonists in CA3 pyramidal neurons of the rat hippocampus. *J Neurosci* 15: 4395–4407, 1995.
- HAJ-DAHMANE S AND ANDRADE R. Ionic mechanism of the slow afterdepolarization induced by muscarinic receptor activation in rat prefrontal cortex. *J Neurophysiol* 80: 1197–1210, 1998.
- HALLIWELL JV. Physiological mechanisms of cholinergic action in the hippocampus. *Prog Brain Res* 84: 255–272, 1990.
- HELL JW, YOKOYAMA CT, BREEZE LJ, CHAVKIN C, AND CATTERALL WA. Phosphorylation of presynaptic and postsynaptic calcium channels by cAMP-dependent protein kinase in hippocampal neurons. *Embo J* 14: 3036–3044, 1995.
- HEMMINGS HC JR, NAIRN AC, MCGUINNESS TL, HUGANIR RL, AND GREENGARD P. Role of protein phosphorylation in neuronal signal transduction. *FASEB J* 3: 1583–1592, 1989.
- HENDEY B, KLEE CB, AND MAXFIELD FR. Inhibition of neutrophil chemokinesis on vitronectin by inhibitors of calcineurin. *Science* 258: 296–299, 1992.
- HERZIG S, MEIER A, PFEIFFER M, AND NEUMANN J. Stimulation of protein phosphatases as a mechanism of the muscarinic-receptor-mediated inhibition of cardiac L-type Ca^{2+} channels. *Pflügers Arch* 429: 531–538, 1995.
- HESCHELER J, KAMEYAMA M, TRAUTWEIN W, MIESKES G, AND SOLING HD. Regulation of the cardiac calcium channel by protein phosphatases. *Eur J Biochem* 165: 261–266, 1987.
- HIDAKA H, WATANABE M, AND KOBAYASHI R. Properties and use of H-series compounds as protein kinase inhibitors. *Methods Enzymol* 201: 328–339, 1991.
- HUANG XY, MORIELLI AD, AND PERALTA EG. Tyrosine kinase-dependent suppression of a potassium channel by the G protein-coupled m1 muscarinic acetylcholine receptor. *Cell* 75: 1145–1156, 1993.
- KLINK R AND ALONSO A. Morphological characteristics of layer II projection neurons in the rat medial entorhinal cortex. *Hippocampus* 7: 571–583, 1997.
- KOSTYUK P AND VERKHRATSKY A. Calcium stores in neurons and glia. *Neuroscience* 63: 381–404, 1994.
- KRAUSE M AND PEDARZANI P. A protein phosphatase is involved in the cholinergic suppression of the Ca^{2+} -activated K^{+} current sI(AHP) in hippocampal pyramidal neurons. *Neuropharmacology* 39: 1274–1283, 2000.
- KRNJEVIC K. Central cholinergic mechanisms and function. *Prog Brain Res* 98: 285–292, 1993.
- LAMBRECHT G, FEIFEL R, WAGNER-RÖDER M, STROHMANN C, ZILCH H, TACKE R, WAELBROECK M, CHRISTOPHE J, BODEKE H, AND MUTSCHLER E. Affinity profiles of hexahydro-sila-difenidol analogues at muscarinic receptor subtypes. *Eur J Pharmacol* 168: 71–80, 1989.
- LOTHMAN EW, BERTRAM EHD, AND STRINGER JL. Functional anatomy of hippocampal seizures. *Prog Neurobiol* 37: 1–82, 1991.
- MALENKA RC, KAUER JA, PERKEL DJ, MAUK MD, KELLY PT, NICOLL RA, AND WAXHAM MN. An essential role for postsynaptic calmodulin and protein kinase activity in long-term potentiation. *Nature* 340: 554–557, 1989.
- MALINOW R, SCHULMAN H, AND TSJEN RW. Inhibition of postsynaptic PKC or CaMKII blocks induction but not expression of LTP. *Science* 245: 862–866, 1989.
- MARSH SJ, TROUSLARD J, LEANEY JL, AND BROWN DA. Synergistic regulation of a neuronal chloride current by intracellular calcium and muscarinic receptor activation: a role for protein kinase C. *Neuron* 15: 729–737, 1995.
- MCCORMICK DA. Actions of acetylcholine in the cerebral cortex and thalamus and implications for function. *Prog Brain Res* 98: 303–308, 1993.
- MICHEL AD, STEFANICH E, AND WHITING RL. Direct labeling of rat M3-muscarinic receptors by [3H]4DAMP. *Eur J Pharmacol* 166: 459–466, 1989.
- MIRONOV SL AND LUX HD. Calmodulin antagonists and protein phosphatase inhibitor okadaic acid fasten the 'run-up' of high-voltage activated calcium current in rat hippocampal neurones. *Neurosci Lett* 133: 175–178, 1991.
- MIZUNO T AND KIMURA F. Medial septal injection of naloxone elevates acetylcholine release in the hippocampus and induces behavioral seizures in rats. *Brain Res* 713: 1–7, 1996.
- MULKEY RM, HERRON CE, AND MALENKA RC. An essential role for protein phosphatases in hippocampal long-term depression. *Science* 261: 1051–1055, 1993.
- MULLER W, PETROZZINO JJ, GRIFFITH LC, DANHO W, AND CONNOR JA. Specific involvement of Ca^{2+} -calmodulin kinase II in cholinergic modulation of neuronal responsiveness. *J Neurophysiol* 68: 2264–2269, 1992.
- NAGAO T, ALONSO A, AND AVOLI M. Epileptiform activity induced by pilocarpine in the rat hippocampal-entorhinal slice preparation. *Neuroscience* 72: 399–408, 1996.
- NICOLL RA, MALENKA RC, AND KAUER JA. Functional comparison of neurotransmitter receptor subtypes in mammalian central nervous system. *Physiol Rev* 70: 513–565, 1990.
- PALMIERI C, KAWASAKI H, AND AVOLI M. Topiramate depresses carbachol-induced plateau potentials in subicular bursting cells. *Neuroreport* 11: 75–78, 2000.
- PARTRIDGE LD, SWANDULLA D, AND MULLER TH. Modulation of calcium-activated non-specific cation currents by cyclic AMP-dependent phosphorylation in neurones of *Helix*. *J Physiol (Lond)* 429: 131–145, 1990.
- PEDARZANI P, KRAUSE M, HAUG T, STORM JF, AND STUHMER W. Modulation of the Ca^{2+} -activated K^{+} current sIAHP by a phosphatase-kinase balance under basal conditions in rat CA1 pyramidal neurons. *J Neurophysiol* 79: 3252–3256, 1998.
- PEDARZANI P AND STORM JF. Evidence that Ca/calmodulin-dependent protein kinase mediates the modulation of the Ca^{2+} -dependent K^{+} current, IAHP, by acetylcholine, but not by glutamate, in hippocampal neurons. *Pflügers Arch* 431: 723–728, 1996.
- RAGGENBASS M, PIERSON P, METZGER D, AND ALBERI S. Action of a metabotropic glutamate receptor agonist in rat lateral septum: induction of a sodium-dependent inward aftercurrent. *Brain Res* 776: 75–87, 1997.
- RAZANI-BOROUJERDI S AND PARTRIDGE LD. Activation and modulation of calcium-activated non-selective cation channels from embryonic chick sensory neurons. *Brain Res* 623: 195–200, 1993.
- REALE V, HALES CN, AND ASHFORD ML. Regulation of calcium-activated nonselective cation channel activity by cyclic nucleotides in the rat insulinoma cell line, CRI-G1. *J Membr Biol* 145: 267–278, 1995.

- RUEGG UT AND BURGESS GM. Staurosporine, K-252 and UCN-01: potent but nonspecific inhibitors of protein kinases. *Trends Pharmacol Sci* 10: 218–220, 1989.
- SHUBA YM, HESSLINGER B, TRAUTWEIN W, McDONALD TF, AND PELZER D. Whole-cell calcium current in guinea-pig ventricular myocytes dialysed with guanine nucleotides. *J Physiol (Lond)* 424: 205–228, 1990.
- SURMEIER DJ, BARGAS J, HEMMINGS HC JR, NAIRN AC, AND GREENGARD P. Modulation of calcium currents by a D1 dopaminergic protein kinase/phosphatase cascade in rat neostriatal neurons. *Neuron* 14: 385–397, 1995.
- SVIRSKIS G AND HOUNSGAARD J. Transmitter regulation of plateau properties in turtle motoneurons. *J Neurophysiol* 79: 45–50, 1998.
- SWARUP G, COHEN S, AND GARBERS DL. Inhibition of membrane phosphotyrosyl-protein phosphatase activity by vanadate. *Biochem Biophys Res Commun* 107: 1104–1109, 1982.
- THOMAS EA, HSU HH, GRIFFIN MT, HUNTER AL, LUONG T, AND EHLERT FJ. Conversion of *N*-(2-chloroethyl)-4-piperidinyl diphenylacetate (4-DAMP mustard) to an aziridinium ion and its interaction with muscarinic receptors in various tissues. *Mol Pharmacol* 41: 718–726, 1992.
- TOSELLI M AND LUX HD. GTP-binding proteins mediate acetylcholine inhibition of voltage dependent calcium channels in hippocampal neurons. *Pflügers Arch* 413: 319–321, 1989.
- VAN DEN ABEELE T, TRAN BA HUY P, AND TEULON J. Modulation by purines of calcium-activated non-selective cation channels in the outer hair cells of the guinea-pig cochlea. *J Physiol (Lond)* 494: 77–89, 1996.
- VILARO MT, MENGOD G, PALACIOS G, AND PALACIOS JM. Receptor distribution in the human and animal hippocampus: focus on muscarinic acetylcholine receptors. *Hippocampus* 3 Spec No: 149–156, 1993.
- WANG YT AND SALTER MW. Regulation of NMDA receptors by tyrosine kinases and phosphatases. *Nature* 369: 233–235, 1994.
- WASTERLAIN CG, FUJIKAWA DG, PENIX L, AND SANKAR R. Pathophysiological mechanisms of brain damage from status epilepticus. *Epilepsia* 34, Suppl 1: S37–S53, 1993.
- WHITE RE, SCHONBRUNN A, AND ARMSTRONG DL. Somatostatin stimulates Ca(2+)-activated K⁺ channels through protein dephosphorylation. *Nature* 351: 570–573, 1991.
- WILSON GF AND KACZMAREK LK. Mode-switching of a voltage-gated cation channel is mediated by a protein kinase A-regulated tyrosine phosphatase. *Nature* 366: 433–438, 1993.
- WILSON GF, MAGOSKI NS, AND KACZMAREK LK. Modulation of a calcium-sensitive nonspecific cation channel by closely associated protein kinase and phosphatase activities. *Proc Natl Acad Sci USA* 95: 10938–10943, 1998.
- YAARI Y AND JENSEN MS. Cholinergic modulation of hippocampal epileptic activity in vitro. *EXS* 57: 150–158, 1989.
- ZHANG L, WEINER JL, AND CARLEN PL. Muscarinic potentiation of IK in hippocampal neurons: electrophysiological characterization of the signal transduction pathway. *J Neurosci* 12: 4510–4520, 1992.