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Selective Potentiation of L-Type Calcium Channel Currents by Cocaine in Cardiac Myocytes

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ABSTRACT

Cocaine use poses a major health problem not only because of the dependence it causes but also because of the generation of life-threatening cardiac arrhythmias following overdose. Elucidating the molecular mechanisms of action of cocaine, therefore, remains a critical step in developing treatment for cocaine addiction and preventing cardiac complications. Although the neurotransmitter transporters are suggested to be primary targets for cocaine, the continued drug-seeking behavior of transporter knock-out mice suggests the involvement of additional mechanisms. Several studies have shown that voltage-gated calcium channel blockers can prevent the behavioral and reinforcing effects of the drug and also cocaine-induced cardiac events, including lethal ventricular fibrillation. However, the role of voltage-gated calcium channels in cocaine-induced responses is not clear. Herein, I show that cocaine, in pharmacological doses, selectively and potently enhances L-type calcium channel currents in isolated rat ventricular myocytes. This potentiation by cocaine is due to an increase and decrease, respectively, in the calcium channel opening and closing rates, with no apparent effects on voltage-dependence or single-channel conductance. The effects of cocaine are rapidly reversible and unaffected by prior ATPγS-induced channel phosphorylation. These results suggest that cocaine directly binds and facilitates the opening of L-type calcium channels. Importantly, elevated intracellular calcium levels via this mechanism triggering second messenger pathways and gene activation may contribute to many of the cardiovascular and central nervous system effects of cocaine.

Cocaine abuse is a major health problem. In the United States alone, it is estimated that 30 to 60 million people have used the drug. Although cocaine is principally abused for its psychostimulant properties, it also can produce undesirable cardiovascular effects. In western countries, the cardiovascular complications of cocaine abuse now account for a major fraction of drug-related emergency room visits and deaths. Within 30 s of cocaine ingestion, peripheral vascular resistance, blood pressure, and heart rate increase and this may induce coronary vasospasm mimicking myocardial infarction. Acute cocaine overdose can generate life-threatening cardiac arrhythmias, whereas chronic cocaine usage can lead to cardiotoxicity due to an overload of intracellular Ca^{2+} via the stimulation of α- and β-adrenergic receptors (Billman, 1995).

In the central nervous system, the major target of cocaine action is the dopaminergic system; cocaine increases extracellular dopamine levels by interacting with the dopamine transporter and acts as a classical uptake blocker (Ritz et al., 1987). Increases in dopamine levels produce dramatic behavioral and biochemical changes. However, other signaling mechanisms are likely to be involved in the development of addiction given the surprising discoveries that mice lacking the dopamine transporter continue to abuse cocaine (Giros et al., 1996; Rocha et al., 1998) and that cocaine-induced place-preference conditioning is maintained in mice lacking both dopamine and serotonin transporters (Sora et al., 1998).

Several studies have shown that voltage-gated Ca^{2+} channel blockers can alter the behavioral and the reinforcing effects of the drug and prevent cocaine-induced events, including lethal ventricular fibrillation (Nahas et al., 1985; Rowbotham et al., 1987; Pani et al., 1990, 1991; Kuzmin et al., 1992; Ansah et al., 1993; Vislobokov et al., 1993; Derlet et al., 1994; Martellotta et al., 1994; Billman, 1995; Rosenzweig-Lipson and Barrett, 1995; Biala and Langwinski, 1996). Use of Ca^{2+}-channel blockers has been proposed as a treatment for the cardiac effects of cocaine intoxication (Nahas et al., 1985). The utility of channel blockers may be due to them inhibiting a direct action of cocaine on the channel, or alternatively, to them compensating for a cocaine-induced signaling pathway that is independent of the channels. In this study, I show that the former is true. I have recorded currents through voltage-gated Ca^{2+} channels and found that cocaine selectively and potently enhances L-type calcium channel currents in ventricular myocytes.

Materials and Methods

Isolated rat ventricular myocytes were prepared as described previously (Mitra and Morad, 1985), and cells were used within 4 to 6 h after isolation. Giga-seal patch clamp techniques (Hamill et al., 1981; Premkumar and Ahern, 1995) were used to record whole-cell and single-channel currents.

Patch electrodes were made from thick-walled borosilicate glass

tubes (Clark Electromedical, Pangbourne, UK), and filled with a solution that contained, unless otherwise indicated, 140 mM K-gluconate, 10 mM KCl, 0.5 mM $MgCl_2$, 10 mM HEPES, 10 mM 1,2-bis(2-aminophenoxy)ethane-*N*,*N*,*N*′,*N*′-tetraacetic acid, 2 mM ATP, 0.25 mM GTP, and pH adjusted to 7.3 with KOH. Electrodes had a resistance of 5 to 15 MΩ. Lower resistance electrodes were used for whole-cell experiments and the higher resistance electrodes were used for single-channel recordings. All experiments were performed at room temperature (22–25°C). Agar-bridge electrodes were used to avoid changes in junction potentials.

For whole-cell recordings, the extracellular solutions had the following composition: 100 mM *N*-methyl-D-glucamine, 40 mM tetraethylammonium, 5 or 10 mM $BaCl_2$ or 5 or 10 mM $CaCl_2$, 10 mM HEPES, and pH adjusted to 7.3 with HCl (for Ba^{2+} or Ca^{2+} currents); and 140 mM NaCl, 2.5 mM KCl, 2 mM $CaCl_2$, 5 mM HEPES, and pH adjusted to 7.3 with NaOH with or without tetrodotoxin (for Na^+ or K^+ currents). Currents were recorded with a current-to-voltage converter (Axopatch 200A; Axon Instruments, Foster City, CA), filtered at 50 kHz, and digitized at 5 kHz with LabView (National Instruments, Autin, TX)-based programs. Capacitance transients were carefully canceled, and series resistance compensation was set at 70 to 80%. In some experiments, the capacity and leak currents were subtracted with a p/4 leak subtraction protocol. Currents were elicited at 30- or 60-s intervals. The cell under voltage clamp was continuously perfused with the control solution flowing from one of the two 300-μm barrels positioned 50 to 100 μm away from the cell. Solutions containing the drugs were applied via the second barrel and the change in flow was initiated by switching a valve that occluded the control solution, and began the flow of the drug solution (complete solution exchange was achieved in <20 ms).

The cell-attached patch configuration of the giga-seal patch clamp technique was used to record single-channel currents from rat ventricular myocytes. The pipette solution contained 110 mM $BaCl_2$, 10 mM HEPES, and pH adjusted to 7.3 by CsOH. The extracellular solution contained 140 mM K-gluconate, 10 mM KCl, 5 mM HEPES, and pH adjusted to 7.3 with KOH. Single-channel currents were filtered at 50 kHz, digitized at 94 kHz (VR-10B; Instrutech, Mineola, NY), and stored on videotape. For analysis, the currents were filtered at 2.5 kHz (−3-db frequency with an 8-pole low pass Bessel filter (Frequency Devices Inc., Haverhill, MA) and digitized at 5 kHz. Continuous data segments were grouped deleting the capacitive transients and analyzed for amplitude and kinetics with a Hidden Markov Model-based technique that idealizes the single-channel current and directly provides the amplitude, P_{open}, mean open and closed times (Chung et al., 1990; Premkumar and Auerbach, 1996).

The drugs were obtained from the following companies: cocaine (Sigma Chemical Co., St.Louis, MO) and nifedipine (Life Technologies, Inc., Grand Island, NY). Nifedipine was dissolved in dimethylsulfoxide and then diluted with the extracellular solutions. Other drugs were dissolved in external solution and the required concentration was made from a stock before the experiment.

Results and Discussion

Whole-cell currents (with Ba^{2+} or Ca^{2+} as the charge carrier) through voltage-gated Ca^{2+} channels were recorded from isolated voltage-clamped rat ventricular myocytes. Currents were activated by depolarizing pulses applied every 30 or 60 s. Figure 1a shows that 1 μM cocaine produced a large increase in myocyte Ba^{2+} current evoked by a step depolarization from −100 to 0 mV. Average of peak currents from two consecutive recordings was calculated. The mean ± S.E. potentiation of Ba^{2+} currents by 1 μM cocaine was 74.5 ± 5.8% of the control (range, 36–123%, n = 16). Cocaine produced a similar enhancement of current when Ca^{2+} was the charge carrier (Fig. 1a, inset; 104 ± 36%, n = 3). Figure 1b shows the time course of cocaine action. Within 2 min after application of cocaine, the peak potentiation was achieved and the effect was fully reversed within 2 min after wash. Fitting the dose versus peak current responses to a Hill equation yielded an EC_{50} value of 274 nM (Fig. 1c). This concentration is relevant because it has been shown that the range of plasma concentrations achieved in recreational use ranges from 1.7 to 3.3 μM (Paly et al., 1982).

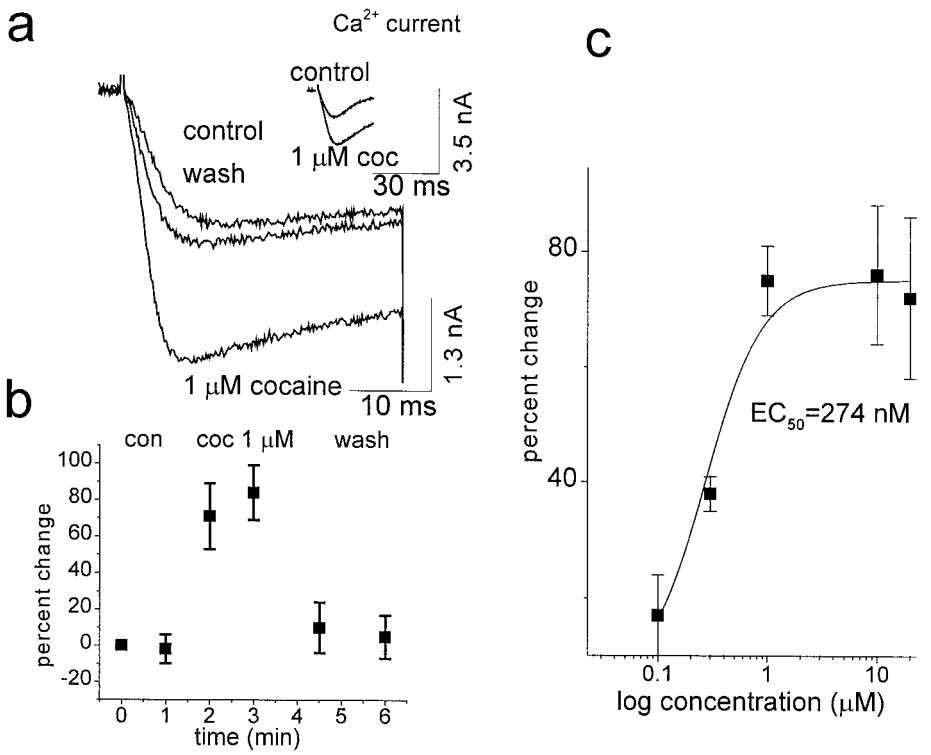

Fig. 1. Potentiation of current through voltage-gated Ca^{2+} channels by cocaine in cardiac myocytes. a, potentiation of Ba^{2+} current by 1 μM cocaine. Currents were evoked by a 30-ms depolarizing pulse from −100 to 0 mV. Inset shows current traces when Ca^{2+} was the charge carrier; 1 μM cocaine potentiated the current to a similar extent. b, time course of cocaine action; the maximum potentiation occurs within 2 min after the application of cocaine and the effect is fully reversed within 2 min after washout. c, dose-response curve yielding an EC_{50} value of 274 nM for the potentiation in ventricular myocytes.

Analysis of the current- or conductance-voltage relationships indicated that cocaine enhances the current without altering the voltage dependence of activation. Figure 2a shows currents evoked by a series of voltage steps before, during, and after washout of 1 μM cocaine. Currents were increased at all membrane potentials. Figure 2d shows the current-voltage relationship from eight different cells with a potentiation of >70%. The current at a given potential is normalized to the maximum current recorded in control conditions. The data points were fitted to the following function:

$$I_{ca}(V) = G_{max} \cdot (V - V_{rev}) / \{1 + \exp[(V_{0.5} - V)/k]\} \quad (1)$$

where V is membrane potential, V_{rev} is the reversal potential for the Ca^{2+} current, G_{max} is the maximum whole-cell conductance, $V_{0.5}$ is the potential of half-maximal activation, and k is the steepness factor.

Table 1 shows the parameters obtained by the best fit to the equation; cocaine approximately doubled the value of G_{max}, without affecting the other parameters. Figure 2e shows the conductance versus voltage curves normalized to the maximum control conductance (conductance was calculated from the reversal potentials shown in Table 1). It is clear from the data that the conductance is doubled in the presence of cocaine. Figure 2f shows G/G_{max} curves with the Boltzmann fits to the data. These observations suggest that cocaine does not interfere with the voltage sensing machinery of the Ca^{2+} channels.

Cocaine also reduced the time to reach the peak, which is clearly seen at hyperpolarized potentials and suggests an increase in the opening rate constant of the channels. In response to a step depolarization from −100 to −10 mV, the time to reach the peak was reduced significantly ($p < .05$; one-way ANOVA) from 11.8 ± 0.58 ms in control conditions to 7.9 ± 0.59 ms ($n = 7$; mean ± S.E.) in the presence of 1 μM cocaine. In addition, analysis of tail currents (Fig. 2c) indicated that the channel-closing rate constant was reduced compared with control conditions. The tail currents could be fitted with a single exponential function with significantly different time constants: 3.0 ± 0.60 ms for control and 5.1 ± 0.65 ms ($n = 6$) in the presence of cocaine (paired t test; $p < .0005$). These data indicate that cocaine accelerates and slows the rate of channel opening and closing, respectively.

Ca^{2+} currents are known to be potentiated by phosphorylation (Cachelin et al., 1983; Bean et al., 1984). To determine whether cocaine action was mediated through channel phosphorylation, a nonhydrolyzable analog of ATP (ATPγS) was included in the recording pipette. ATPγS alone produced a time-dependent increase in the basal current level that is presumably as a result of basal kinase activity. However, subsequent application of 1 μM cocaine reversibly potentiated the current to the same extent as seen without ATPγS (96.9%; Fig. 2b). Thus, this result indicates that cocaine acts independently of phosphorylation and taken together with the rapid onset of the response suggests that cocaine acts directly on the Ca^{2+} channel or an associated protein. Although, it remains possible that other signaling pathways, for example, G protein interactions, are involved.

To confirm the effects seen in the whole-cell experiments and to further understand the mechanism of action of cocaine, single-channel Ca^{2+} currents were recorded in cell-attached patches from ventricular myocytes. In these experiments, the bathing K^+ concentration was first raised to 140 mM to bring the resting membrane potential close to 0 mV. Thereafter, a step depolarization from −70 to +30 mV activated channels in 15 out of 50 patches. Patches containing multiple channels showed much higher activity when the pipette contained 1 μM cocaine (Fig. 3b) compared with controls (Fig. 3a). Data from patches containing a single L-type

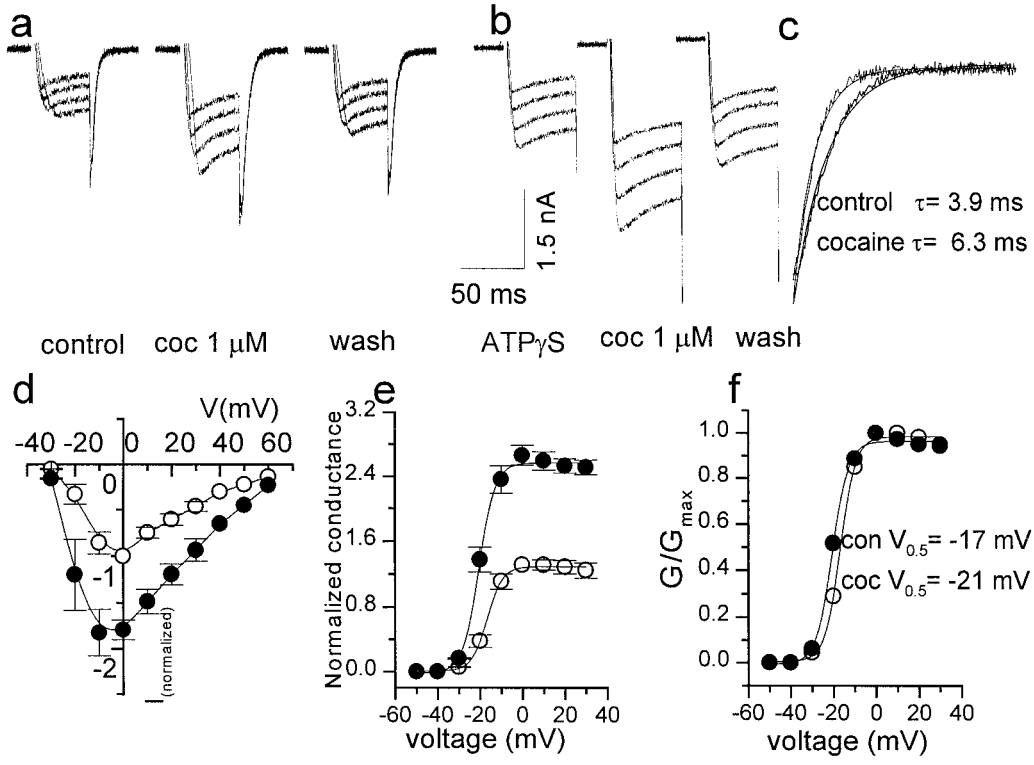

Fig. 2. Analysis of the potentiation of current through voltage-gated Ca^{2+} channels by cocaine. a, calcium currents recorded at different membrane potentials from a holding potential of −100 to +10 mV and thereafter in 10-mV increments, showing clear potentiation at all voltages. b, potentiation of Ca^{2+} current by cocaine when the channels were phosphorylated by the addition of ATPγS in the pipette. c, tail currents in control and after application of cocaine. The data are fitted by a single exponential function (smooth line). The time constant (τ) for channel closing is prolonged in the presence of cocaine. d, normalized current-voltage relationship under control conditions (○) and in the presence of cocaine (●). The continuous lines are the fit to eq. 1. e, normalized conductance is plotted as a function of voltage. The conductance is doubled in the presence of cocaine. f, G/G_{max} curves fitted with a Boltzmann equation (see text). Cocaine produced negligible changes in the values of $V_{0.5}$ and k.

Ca^{2+} channel (without superimposed openings) were analyzed in detail. The single channel slope conductance was estimated to be 23 pS by plotting I–V curves and fitting the data points to a linear function. This value is consistent with previously published conductance for L-type channels (Brum et al., 1984). Figure 3, c and d show single-channel current traces, and all point amplitude histograms under control conditions and in the presence of 1 μM cocaine. Cocaine approximately doubled the open probability of the channel, from 0.16 ± 0.03 (n = 3) in control to 0.35 ± 0.08 (n = 4) in 1 μM cocaine without changing the single-channel current amplitude: 0.84 ± 0.03 pA in control and 0.89 ± 0.07 pA in the presence of cocaine. Cocaine also increased the open time of channels from 1.8 ± 0.1 ms in control conditions to 3.7 ± 0.6 ms in the presence of 1 μM cocaine. These results indicate that cocaine increases the open probability and open time of L-type Ca^{2+} channels.

To identify the type of Ca^{2+} channel potentiated by cocaine and to test the selectivity of the response, the L-type Ca^{2+} channel blocker nifedipine was used. In six cells, application of 25 to 50 μM nifedipine completely blocked Ba^{2+} current, indicating that cocaine potentiated L-type Ca^{2+} channel current in ventricular myocytes. At higher concentrations, cocaine is known to block Na^+, K^+, and Ca^{2+} channel currents and produce a local anesthetic action. In this study, <2 μM cocaine selectively potentiated L-type Ca^{2+} current and had no effect on voltage-gated Na^+ and K^+ currents. At higher concentrations, all of these currents were blocked (data not shown) (Crumb and Clarkson 1990; Stewart et al., 1993; Renard et al., 1994). Furthermore, cocaine also was devoid of potentiating or inhibiting actions on *N*-methyl-D-aspartate and γ-aminobutyric acid receptor-mediated currents (data not shown). These observations indicate that at lower concentrations, cocaine selectively potentiates L-type Ca^{2+} channels but at higher concentrations it produces a nonselective block of cationic conductances. The selective potentiating effect of cocaine may be due to its binding (Calligaro and Eldefrawi, 1987) to a specific domain in the Ca^{2+} channel that stabilizes the open state of the channel.

This study shows that cocaine potently enhances L-type Ca^{2+} channel currents in cardiac myocytes. This novel action of cocaine occurs at low concentrations and is likely to be pharmacologically important because these channels play important roles in Ca^{2+} homeostasis in many tissues. In particular, L-type channels contribute to cardiac action potentials, synaptic plasticity, and hormonal secretion. In the heart, low concentrations (3 μM) of cocaine prolong action potentials and produce positive inotropy and these actions may be directly explained by enhancement of L-type Ca^{2+} current shown herein. In contrast, higher concentrations (30 or 100 μM) of cocaine shorten action potentials probably due to an inhibition of Ca^{2+} current and a more generalized inhibition of cationic conductances (Clarkson et al., 1996). Chronic cocaine abuse can lead to severe cardiomyopathy and ventricular hypertrophy, which are probably due to intracellular Ca^{2+} overload (Billman, 1995). Indeed, Ca^{2+}-dependent activation of the phosphatase calcineurin, which in turn activates several transcriptional pathways, has been implicated in ventricular hypertrophy (Molkentin et al., 1998). Flunarizine, a Ca^{2+} overload antagonist that does not interact with Ca^{2+} channels is effective in preventing cocaine- but not reentry-induced cardiac arrhythmias, suggesting that Ca^{2+} overload is responsible for cocaine-induced cardiac arrhythmias (Vos et al., 1990). The cardioprotection afforded by the Ca^{2+}-channel blockers suggest that the cocaine-induced potentiation of L-type channels described herein may significantly contribute to intracellular Ca^{2+} overload.

Importantly, the activation of L-type Ca^{2+} channels by cocaine may not only be restricted to the heart but also may occur in other tissues. In particular, calcium entry via neuronal L-type Ca^{2+} channels has been implicated in complex events such as gene activation and synaptic plasticity (Murphy et al., 1991; Bading et al., 1993; Ghosh and Greenberg, 1995; Nestler and Aghajanian, 1997; Deisseroth et al., 1998). Thus, increases in the intracellular Ca^{2+} by cocaine acting on these cellular signaling pathways may contribute to the reinforcing and drug-seeking behavior. It is interesting that the block of Ca^{2+} flux by ibogaine, a putative antiaddictive drug, appears to be due to a direct binding to L-type Ca^{2+} channels, as ibogaine inhibits the binding of the dihydropyridine, [^{3}H]isradipine (Popik et al., 1995). This also lends further support to the notion that cocaine directly interacts with L-type Ca^{2+} channels.

TABLE 1

Analyses of Current Voltage and Conductance Voltage Relationships

$V_{0.5}$ is the potential of half-maximal activation, G_{max} is the maximum whole-cell conductance, V_{rev} is the reversal potential for the Ca^{2+} current, and k is the steepness factor.

	$V_{0.5}$	G_{max}	V_{rev}	k
	mV	*nS*	*mV*	*mV/e-fold*
Control	−16.76 ± 0.58	1.29 ± 0.06	67.27 ± 1.92	3.77 ± 0.43
Cocaine	−20.44 ± 0.35	2.57 ± 0.08	67.40 ± 1.41	3.76 ± 0.36

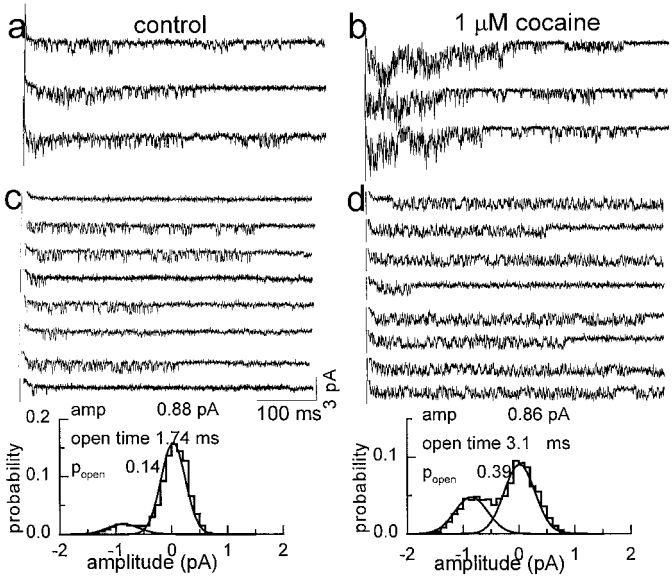

Fig. 3. Effect of cocaine on single voltage-gated Ca^{2+} channels. Single channel currents were recorded in cell-attached patches with the pipette solution containing 110 mM $BaCl_2$ with or without 1 μM cocaine. Channels were activated by a 500-ms depolarizing pulse from −70 to +30 mV. a and b, potentiation by cocaine of Ca^{2+} currents in multichannel patches. c and d, in single-channel patches, cocaine increased the open probability and open time of the channel. Continuous records of eight sweeps are shown. All point amplitude histograms and the Gaussian fits to the data are shown below. Hidden Markov Model-based techniques were used for analysis of amplitude, P_{open}, and open times. It is clear that cocaine increases the open probability that results from an increase in the open time, and a decrease in the closed time of Ca^{2+} channels.

In the ribbon synapse of the retina and many cells of the endocrine system, secretion of neurotransmitters and neuropeptides are modulated by Ca^{2+} influx through L-type Ca^{2+} channels (Elhamdani et al., 1998). Accordingly, cocaine should enhance secretion in these tissues, given the potentiation of L-type channels described in this study. One of the most profound and immediate effects after cocaine ingestion is an increase in epinephrine secretion. Cocaine also increases secretion of growth hormone, corticotropin-releasing hormone, and adrenocorticotropic hormone from the anterior pituitary and oxytocin/vasopressin from the neurohypophysis. Consistent with cocaine modulating neuropeptide secretion is the higher incidence of neuroendocrine abnormalities such as gynecomastia and premature births in chronic users (Gold, 1993). Thus, the direct activation of L-type channels by cocaine is likely to play an important role in many of the actions in the cardiovascular and central nervous systems.

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