Pharmacological Reduction of Small Conductance Calcium-Activated Potassium Current (SK) Potentiates the Excitatory Effect of Ethanol on Ventral Tegmental Area Dopamine Neurons

MARK S. BRODIE, MAUREEN A. MCELVAIN, E. BRADSHAW BUNNEY, and SARAH B. APPEL
Departments of Physiology and Biophysics (M.S.B., M.A.M., S.B.A.) and Emergency Medicine (E.B.B.), University of Illinois at Chicago, College of Medicine, Chicago, Illinois
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ABSTRACT
Dopaminergic neurons in the ventral tegmental area (VTA) are important for the rewarding properties of drugs of abuse, including ethanol. We previously demonstrated that ethanol excites VTA neurons and that ethanol reduces the amplitude of the after hyperpolarization (AHP) that follows spontaneous action potentials. Because the small conductance calcium-activated potassium current (SK) is a component of the AHP of VTA neurons, we assessed the effect of the SK blockers apamin and d-tubocurarine (d-TC) on the action of ethanol on dopaminergic VTA neurons with intracellular and extracellular recording in rat brain slices. Apamin (1–200 nM) and d-TC (100 and 400 μM) caused concentration-dependent reductions in the AHP amplitude. Ethanol (80 mM) caused a small reduction in the AHP. In the presence of apamin (40 nM), ethanol (80 mM) caused a much larger reduction in AHP amplitude. Extracellular studies showed that apamin (20, 40, and 100 nM) and d-TC (400 μM) had no significant effect on the spontaneous firing rate of dopaminergic VTA neurons but enhanced the potency of ethanol to increase their firing rate. These results indicate that the ethanol-induced reduction of the AHP and excitation of VTA neurons is not due to a reduction in SK current. Furthermore, blockade of SK current by apamin or d-TC enhances the excitatory effect of ethanol on dopaminergic VTA neurons. These data suggest that the amount of SK current present affects the sensitivity of dopaminergic VTA neurons to ethanol excitation and that neurotransmitters that reduce SK current may increase the reward potency of ethanol.

Dopaminergic systems of the brain are of critical importance for the rewarding effects of drugs of abuse, including ethanol. The mesolimbic/mesocortical dopamine pathways maintain self-administration of drugs of abuse like cocaine, opiates, and ethanol (Wise, 1987). For example, in behavioral experiments, dopaminergic antagonists reduce self-administration of ethanol in a two-bottle choice paradigm (Pfeffer and Samson, 1986; Samson et al., 1990). Further analysis of the action of dopamine agonists and antagonists on ethanol preference suggests that ethanol preference is related to increased dopaminergic tone (Samson et al., 1990; Hodge et al., 1992). The ventral tegmental area (VTA) is the source of dopaminergic innervation of the nucleus accumbens and is likely to be an important site of action of ethanol on the reward circuitry of the brain (Wise, 1987). The observation that rats will self-administer ethanol directly into the VTA (Gatto et al., 1994; Rodd et al., 1998) suggests that the VTA is a critical area for the rewarding properties of ethanol.

We have demonstrated that ethanol excites dopaminergic VTA neurons in a concentration-dependent manner over a pharmacologically-relevant concentration range (20–200 mM) in extracellular single-unit studies in brain slices (Brodie et al., 1990). Our intracellular studies in brain slices have shown that ethanol reduces the after hyperpolarization (AHP) that follows the spontaneous action potential in VTA neurons, and we have suggested that this effect may underlie or contribute to the ethanol-induced increase in spontaneous firing rate (Brodie and Appel, 1998). Four potassium currents are present in mesencephalic dopamine neurons (Silva et al., 1990) that could contribute to generation of the AHP: A current, delayed rectifier, and two types of calcium-dependent potassium currents [small conductance (SK) and big conductance (BK) calcium-activated potassium channels].

ABBREVIATIONS: aCSF, artificial cerebrospinal fluid; AHP, after hyperpolarization; d-TC, d-tubocurarine; SK, small conductance calcium-activated potassium channel; VTA, ventral tegmental area.
The potassium currents that mediate different components of the AHP have been defined in sensorimotor cortical neurons (Schwindt et al., 1988b) and locus ceruleus neurons (Osmancic et al., 1990; Osmanovic and Shefner, 1993), but this type of analysis of the contribution of multiple potassium currents has not yet been done for mesencephalic dopamine neurons. However, the effects of apamin, a bee venom toxin that selectively blocks SK channels (Castle et al., 1989), has been studied on the AHP of these dopamine cells. Apamin (100 nM to 1 μM) reduces a kinetically distinct portion of the AHP in mesencephalic dopamine neurons (Shepard and Bunney, 1991; Seutin et al., 1993). High concentrations (100 μM to 2 mM) of the nicotinic receptor antagonist d-tubocurarine (d-TC) also block SK channels (Dun et al., 1986; Osmanovic and Shefner, 1993). In the present study, we assessed the effect of apamin on the ethanol-induced reduction in the AHP amplitude of dopaminergic VTA neurons with intracellular recording. Furthermore, to determine the importance of SK current in the excitatory effects of ethanol, we tested the effects of apamin and d-TC on ethanol-induced increases in the firing rate of VTA neurons measured with extracellular single unit recording. Some of these results have been previously reported in abstract form (Brodie et al., 1997, 1998).

Materials and Methods

Brain Slice Preparation. Brain slices from male Fischer 344 rats (90–150 g) containing the VTA were prepared as described previously (Brodie and Dunwiddie, 1987; Brodie et al., 1990). Animals used in this study were treated in strict accordance with the National Institutes of Health “Guide for the Care and Use of Laboratory Animals.” Briefly, rats were sacrificed by cervical dislocation, and the brain was rapidly removed from the cranium and kept chilled and moist during dissection. No general anesthetic agent was used, to avoid possible cross-reactivity with ethanol. The tissue was blocked coronally to contain the VTA and substantia nigra; cerebral cortices and a portion of the dorsal mesencephalon were removed from the block. The tissue block was attached to the vibratome chuck using cyanoacrylic glue and submerged in chilled artificial cerebrospinal fluid (aCSF). Coronal sections (400 μm thick) were cut, and the tissue was placed directly in the recording chamber. Equilibration time of at least 1 h was allowed after placement of tissue in the recording chamber before electrodes were placed in the tissue. The slice was covered with medium and a superfusion system maintained the flow of medium at 2 ml/min; the temperature in the recording chamber was kept at 35°C. The VTA was clearly visible in the fresh tissue as a gray area medial to the darker substantia nigra and separated from the nigra by white matter. Recording electrodes were placed in the VTA under visual control. The flow rate of fluid to the recording chamber was continuously monitored with a flowmeter, and adjustable valves were used to keep the rate constant. The small volume chamber used in this study permitted the rapid application and washout of drug solutions. Because the slice was submersed in aCSF in the recording chamber, applied agents reach equilibrium in the tissue quickly (2–3 min). The composition of the aCSF in these experiments was 126 mM NaCl, 2.5 mM KCl, 1.24 mM NaH2PO4, 2.4 mM CaCl2, 1.3 mM MgSO4, 26 mM NaHCO3, and 11 mM glucose; the aCSF was saturated with 95% O2/5% CO2 (pH 7.4).

Cell Identification. Dopamine neurons have been shown to have electrophysiological characteristics very different from nondopaminergic neurons in the mesencephalon (Grace and Bunney, 1984b; Lacey et al., 1989). Only the neurons that were anatomically located within the VTA and that conformed to the criteria for putative dopamine-type neurons established in the literature and in this laboratory (Mueller and Brodie, 1989; Lacey et al., 1989) were studied. These criteria include broad action potentials and slow spontaneous firing rate (0.5–5 Hz) with a regular interspike interval. Because of the strong linkage in the literature between dopamine and reward and because the neurochemical identity of the “nondopamine” neurons in this region has not been established, these other cells were not studied.

Extracellular Recording. Extracellular recording electrodes were made from 1.5-mm diameter glass tubing with filament and were filled with 0.9% NaCl. Tip resistance of the microelectrodes ranged from 4 to 8 MΩ. Frequency of firing was determined with a window discriminator and ratemeter, displayed on a chart recorder, and stored for analysis on a computerized data acquisition system. Each neuron served as its own control; drug responses were quantified as the mean change in firing rate (normalized as a percentage of control) over a 1-min interval during the peak of the drug response. This normalization controlled for minor changes in firing rate that occur spontaneously over time. This method has been used by us in the past (Brodie et al., 1990) and has proved to be reliable. When large changes in the baseline firing rate occurred, this percentage was judged to be unreliable and these data were not used.

Intracellular Recording. Electrodes were fabricated from glass micropipettes (1.0 mm o.d., fiber filled) containing 2 M KCl (resistances from 60–100 MΩ). Voltage recordings and current injection were accomplished through the same electrode with an Axoclamp 2A amplifier (Axon Instruments, San Rafael, CA), and the bridge balance was checked often throughout the experiments and adjusted when necessary. Current and voltage were monitored on a storage oscilloscope and on a rectilinear pen recorder. In most intracellular experiments, the effect of apamin or d-TC on spontaneous action potentials was assessed. In some experiments, the resting membrane potential was held 5 to 10 mV below threshold under current clamp conditions, and then spikes were evoked by passing a positive current pulse of 0.1-ms duration and of sufficient amplitude to reliably evoke an action potential. Action potentials (spontaneous or evoked) were digitized and stored on a computer for later averaging and analysis with pClamp software and a TL-1 DMA interface (Axon Instruments).

Drugs were added to the aCSF in the fluid delivery tubing by means of a calibrated infusion pump from stock solutions 100 to 1000 times the desired final concentrations. The addition of drug solutions to the aCSF was performed in such a way as to permit the drug solution to mix completely with the aCSF before this mixture reached the recording chamber. Final concentrations were calculated from aCSF flow rate, pump infusion rate, and concentration of drug stock solution. A stock solution of 95% USP ethanol was used in the pump, and infusion of ethanol never exceeded 1% of the flow rate of the aCSF. Ethanol was administered for 5 to 8 min before measurements were made to ensure equilibration of the full ethanol concentration in the recording chamber.

The behaviorally active range for blood ethanol concentrations in the rat extends from about 40 mM (sedation) to 90 mM (loss of righting reflex) (Majchrowicz and Hunt, 1976); the lethal blood ethanol concentration in rats is about 200 mM (LD50 = 202 mM; Haggard et al., 1940). The present study examined ethanol concentrations in the range of 20 to 120 mM, pharmacologically relevant, sublethal concentrations in the rat.

Because apamin does not wash out of the preparation quickly, lower concentrations of apamin were always tested (in the absence and presence of ethanol) before higher concentrations of apamin were administered.

Statistical Analysis. Averaged numerical values are expressed as the mean ± S.E.M. Parameters were measured in each neuron before and during administration of ethanol, with each cell serving as its own control. To control for variation in the spontaneous firing rates before ethanol administration among different VTA neurons, increases in firing rate caused by ethanol were expressed as percent change over the control firing rate (see legend of Fig. 3 for formula).
Differences in ethanol-induced excitation produced by either apamin or d-TC were evaluated with a two-way ANOVA.

**Results**

**Intracellular Studies.** The VTA neurons chosen for this intracellular study (n = 21) were identified as dopaminergic according to electrophysiological criteria (see Materials and Methods) and had stable resting membrane potentials (−45 to −65 mV) and input resistances of 100 to 350 MΩ. All neurons exhibited time-dependent inward rectification, which is characteristic of dopaminergic neurons in the VTA and is not seen if the cells are in poor condition. In addition, all neurons showed overshooting spontaneous and/or evoked action potentials with prominent AHPs. Most of the neurons (67%) were spontaneously active.

**Apamin Enhances Ethanol-Induced Reduction in the Spike AHP of Dopaminergic VTA Neurons.** In a previous study from our laboratory (Brodie and Appel, 1998), we demonstrated that ethanol (40–160 mM) reduced the AHP that follows spontaneous action potentials in 74% of dopaminergic VTA neurons tested. In the VTA neuron illustrated in Fig. 1A, 80 mM ethanol caused a small reduction in the amplitude of the AHP. When 40 mM apamin was subsequently applied to the same VTA neuron, it substantially reduced the amplitude of the AHP (Fig. 1B). When 80 mM ethanol was retested in the presence of 40 nM apamin, it produced a greater reduction in the AHP (compare Fig. 1A with Fig. 1C). A similar enhancement by 40 nM apamin of the reduction in AHP amplitude by 80 mM ethanol was seen in five of five dopaminergic VTA neurons tested.

**Apamin and d-TC Cause Concentration-Dependent Reductions in the Spike AHP.** Concentrations of apamin from 1 to 200 nM caused concentration-dependent reductions in the amplitude of the spike AHP (n = 10). Apamin reduced both AHPs that followed spontaneous action potentials (n = 8) and AHPs that followed action potentials evoked by brief depolarizing current pulses (n = 7). Figure 2A shows the concentration-dependent reduction by apamin (20–200 nM) of AHPs that follow spontaneous action potentials recorded intracellularly from a representative dopaminergic VTA neuron. Note that even 20 nM apamin caused a very large reduction in AHP amplitude at 100 ms on the time axis and that in this cell the peak of the AHP appears to move to an earlier point when the SK component is blocked by apamin. This illustrates that apamin blocks a kinetically distinct phase of the AHP in dopaminergic VTA neurons that lasts about 200 ms after the downstroke of the AHP.

It is not possible to determine whether complete block of the SK component was achieved in these experiments because it is likely that other potassium currents are also active during this phase of the AHP (Silva et al., 1990). Figure 2A illustrates, however, that a doubling of the apamin concentration from 100 to 200 nM caused only a small additional reduction in AHP amplitude, suggesting that apamin block of the SK component of the AHP was approaching a maximal level in our experiments.

Concentrations of d-TC from 50 to 400 μM caused concentration-dependent reductions in the amplitude of the spike AHP (n = 7). d-TC reduced both AHPs that followed spontaneous action potentials (n = 3) and AHPs that followed action potentials evoked by brief depolarizing current pulses (n = 4). Figure 2B shows the concentration-dependent reduction by d-TC (50–400 μM) of AHPs that follow evoked action potentials recorded intracellularly from a representative dopaminergic VTA neuron. Subsequent application of 40 nM...
apamin from 20 to 100 nM produced no significant change in d.

Concentrations of d-TC (about 10^4-fold higher) were required to cause reductions in AHP amplitude of comparable magnitudes to those caused by apamin. This observation is consistent with the reported high affinity of SK channels present in the mesencephalon for apamin (Köhler et al., 1996) and the relatively low affinity of d-TC for SK channels (Ishii et al., 1997).

Figure 2. Blockade of SK current reduces the AHP amplitude. A, apamin causes a concentration-dependent reduction in the amplitude of the AHP of dopaminergic VTA neurons. Each waveform represents the average of 10 intracellularly recorded spontaneous action potentials. The positive portion of the action potential has been truncated to show the AHP in more detail. Waveforms were collected before (Control) and at least 10 min after the administration of each of four concentrations of apamin (20, 40, 100, and 200 nM) to the same VTA neuron. This effect of apamin was not reversible with washing. B, d-TC reduces the amplitude of the AHP of dopaminergic VTA neurons in a concentration-dependent manner. Each waveform represents the average of 10 intracellularly recorded action potentials. In this experiment, action potentials were evoked by injection of a brief depolarizing pulse through the recording electrode. The negative capacitive component of the stimulus artifact can be seen at about 60 ms. The positive portion of the action potential is truncated to show the AHP in more detail. Waveforms were collected before (Control) and at least 10 min after the administration of each of four concentrations of d-TC (50, 100, 200, and 400 μM) to the same VTA neuron. Subsequent application of 40 nM apamin caused no further reduction in AHP amplitude.

Apamin and d-TC Potentiate Ethanol-Induced Excitation of Dopaminergic VTA Neurons. In the present study, as in previous studies from our laboratory (Brodie et al., 1990; Brodie and Appel, 1998), ethanol (20–120 mM) produced concentration-dependent excitation of VTA neurons. Figure 3A shows that superfusion of the slice with ethanol (80 and 120 mM) increased the firing rate of spontaneous action potentials recorded extracellularly from a typical dopaminergic VTA neuron and that these effects reversed with washout of ethanol. In the presence of 20 nM apamin (Fig. 3B) and 40 nM apamin (Fig. 3C), these same concentrations of ethanol caused greater increases in firing rate in the same VTA neuron. Percentage increases in firing rate caused by ethanol were calculated according to the formula

The mean firing rate of VTA neurons tested with 20 nM apamin, before the application of apamin, was 1.32 ± 0.14 Hz, and in the presence of apamin, it was 1.46 ± 0.20 Hz (n = 7, paired t test, N.S., P > .05). Similarly, the mean firing rate of those cells tested with 40 nM apamin was 1.46 ± 0.08 Hz before apamin application and 1.51 ± 0.15 Hz in the presence of apamin (n = 10, paired t test, N.S., P > .05). The mean firing rate of the cells tested with 100 nM apamin was 1.48 ± 0.13 Hz before apamin and 1.57 ± 0.14 Hz in the presence of apamin (n = 11, paired t test, N.S., P > .05). Occasional bursts of action potentials were seen in the presence of 40 nM (1 of 10 cells) and 100 nM (3 of 11 cells) apamin; however, the interval between bursts was quite variable (10 s to 20 min) in the neurons that did show bursting. Bursting was not seen in the presence of 20 nM apamin. In one neuron that did not exhibit bursting in apamin alone, some bursting activity was observed in the presence of ethanol and apamin in the experiments described below.

Administration of d-TC (100 and 400 μM) caused a small increase or no change, respectively, in the firing rate of dopaminergic VTA neurons. The mean firing rate of VTA neurons tested with 100 μM d-TC, before d-TC application, was 1.32 ± 0.12 Hz, and in the presence of d-TC, it was 1.58 ± 0.14 Hz (n = 8, paired t test, P < .005). In contrast, the mean firing rate of those cells tested with 400 μM d-TC was 1.46 ± 0.18 Hz before d-TC application and 1.87 ± 0.25 Hz in the presence of d-TC (n = 7, paired t test, N.S., P > .05). Bursting was not seen in the presence of either concentration of d-TC, and no bursting was elicited by ethanol in the presence of d-TC in the experiments described below.
5A shows that no significant enhancement of ethanol excitation was seen in the presence of 100 μM d-TC (n = 8, two-way ANOVA, P > .05). In contrast, 400 μM d-TC (Fig. 5B) produced a significant enhancement in the excitatory effect of ethanol on dopaminergic VTA neurons (n = 7, two-way ANOVA, P < .002). In each case, the effect of ethanol concentration was also statistically significant (P < .001).
centration was also statistically significant \((P < .001)\), indicating that ethanol excitation was concentration dependent.

*d*-TC, but Not Apamin, Blocks Nicotine-Induced Excitation of Dopaminergic VTA Neurons. In addition to its blockade of SK channels, a better known effect of *d*-TC is to block nicotinic cholinergic receptors (Kuba et al., 1989). Because VTA neurons are known to have nicotinic receptors (Brodie, 1991), we considered the possibility that the potentiation of ethanol by *d*-TC and apamin might be related to nicotinic receptor blockade. Figure 6A shows that superfusion of the slice with nicotine (250 and 100 nM) increased the firing rate of spontaneous action potentials recorded extracellularly from a typical dopaminergic VTA neuron and that these effects reversed with washout of nicotine. Figure 6B shows that when these concentrations of nicotine were tested in the same VTA neuron in the presence of 40 nM apamin, a similar excitation was seen. By contrast, bath application of 100 \(\mu\)M *d*-TC completely blocked the excitatory action of nicotine.

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\text{Percentage change in firing rate} = \frac{FR_n - FR_c}{FR_c} \times 100
\]

where \(FR_n\) is the firing rate in the presence of nicotine and \(FR_c\) is the control firing rate before nicotine application. A, before apamin or *d*-TC administration, nicotine increased the firing rate of this neuron by 89.0% (250 nM) and 45.3% (100 nM). B, in the presence of 40 nM apamin, nicotine increased the firing rate of this neuron by 88.1% (250 nM) and 42.6% (100 nM). C, in the presence of 100 \(\mu\)M *d*-TC, nicotine increased the firing rate of this neuron by 2.2% (250 nM) and −7.9% (100 nM). Apamin and *d*-TC alone caused little change in the basal firing rate. Note that the excitation by nicotine was completely blocked by *d*-TC but was unaffected by apamin.
nicotine on this VTA neuron (Fig. 6C). Similar experiments were repeated in six dopaminergic VTA neurons, and the percentage increase in firing rate caused by nicotine was calculated (see Fig. 6 legend for formula). Under control conditions, the mean percentage increase in firing rate was 66.8 ± 10.2% for 250 nM nicotine (n = 6) and 37.9 ± 8.7% for 100 nM nicotine (n = 5). In the presence of 40 nM apamin, the mean percentage increase in firing rate in response to nicotine was 75.3 ± 13.8% for 250 nM nicotine (n = 5) and 47.9 ± 5.4% for 100 nM nicotine (n = 4). In the presence of 50 to 100 μM d-TC, the mean percentage change in firing rate in response to nicotine was 5.8 ± 1.7% for 250 nM nicotine (n = 5) and 1.1 ± 2.3% for 100 nM nicotine (n = 4). A two-way ANOVA indicated a statistically significant effect of nicotine concentration (P < .01) and antagonist condition (P < .001). Student-Newman-Keuls posthoc tests indicated that the nicotine excitation was concentration dependent (P < .05) and that the effect of d-TC to block the nicotine excitation was significant (P < .05) but that apamin had no effect (P > .05).

Discussion

Our previous extracellular single-unit studies in brain slices have demonstrated that ethanol excites dopaminergic VTA neurons in a concentration-dependent manner over a pharmacologically relevant concentration range (20–200 mM) (Brodie et al., 1990). Our previous intracellular studies have shown that ethanol reduces the AHP that follows the spontaneous action potential in VTA neurons, and we have suggested that this effect may underlie or contribute to the ethanol-induced increase in spontaneous firing rate (Brodie and Appel, 1998). Because SK current contributes to the AHP, in the present study we tested the effects of SK blockade (with apamin or d-TC) on ethanol effects on firing rate and AHP amplitude. Apamin potentiated ethanol excitation of dopaminergic neurons and the ethanol-induced reduction in AHP amplitude. Potentiation of ethanol-induced excitation was also seen in the presence of a high concentration of d-TC, which caused a substantial reduction of the SK component of the AHP, but not with a lower concentration of d-TC, which caused a more modest reduction in SK current. These results suggest that the amount of SK current present in dopaminergic VTA neurons can affect the magnitude of the response of these reward neurons to ethanol.

Intracellular recording was used to compare the effect of apamin, d-TC, and ethanol on the AHP amplitude of dopaminergic VTA neurons in brain slices. Both ethanol and apamin reduced the AHP that follows the spontaneous spike, but the change in the shape of the AHP differed for the two drugs. Furthermore, block of SK with apamin did not occlude the ethanol-induced reduction in the AHP; in fact, this effect was potentiated. This occurred despite the fact that 40 to 100 nM apamin caused nearly complete block of the SK component of the AHP. These data indicate that the ethanol-induced reduction of the AHP is not due to a reduction in SK current but that another potassium current may be involved. Ethanol (20–120 mM) caused a concentration-dependent increase in spontaneous firing rate recorded extracellularly, as previously observed. Apamin and d-TC, in concentrations that caused large reductions in the SK component of the AHP, caused little or no increase in spontaneous firing rate, whereas ethanol caused smaller reductions in AHP amplitude but significantly increased firing rate. The fact that apamin and d-TC did not mimic and/or occlude the ethanol-induced increase in spontaneous firing rate indicates that the ethanol excitation of dopaminergic VTA neurons is not due to a reduction in SK current. The observation that ethanol-induced reduction of the AHP is enhanced by apamin is consistent with a relationship between reduced SK and the potentiation of ethanol-induced excitation seen in the extracellular experiments.

The higher concentration of d-TC (400 μM) tested in this study caused a reduction in the SK component of the AHP similar in magnitude to the reduction by apamin (20–100 nM) and, like apamin, enhanced the excitatory action of ethanol. In contrast, 100 μM d-TC caused a much smaller reduction in the AHP and did not significantly potentiate ethanol excitation of VTA neurons. These data suggest that a substantial reduction of SK current is necessary for potentiation of ethanol-induced excitation. The fact that the concentration of d-TC required for a comparable reduction in AHP amplitude and ethanol potentiation was several orders of magnitude higher than the apamin concentration is consistent with the much higher potency of apamin to block SK current than d-TC reported in the literature (Park, 1994; Köhler et al., 1996). Three isoforms of SK channels (SK1, SK2, and SK3) have been identified, which differ in their sensitivity to block by apamin and d-TC (Köhler et al., 1996). The SK3 isoform has been shown to be present in the rat VTA with in situ hybridization mapping (Köhler et al., 1996), and the apamin sensitivity of SK3 channels (50% block with 2 nM apamin) (Ishii et al., 1997) is similar to the concentration dependence for apamin block of the SK component of the AHP of VTA neurons in the present study. Given the putative role of the VTA/mesolimbic dopamine system in schizophrenia, it is intriguing that a human form of the SK3 gene, hSKCa3, has a polymorphic CAG repeat that is significantly longer in schizophrenic patients than in normal control individuals (Chandy et al., 1998). Genetic defects in SK channels could potentially confer enhanced responsiveness of dopamine reward neurons to alcohol; therefore, it is possible that SK gene abnormalities will be found in alcoholic patients, as well.

As described above, both apamin (20–100 nM) and d-TC (400 μM) potentiated ethanol-induced excitation of dopaminergic VTA neurons. This effect was not mediated by the well known action of d-TC to block nicotinic acetylcholine receptors because 100 μM d-TC, which did not potentiate ethanol excitation, was sufficient to block the action of applied nicotine, and apamin (40 nM) had no effect on nicotine-induced excitation (Fig. 6). This observation is consistent with previous reports in bullfrog sympathetic ganglion cells showing that blockade of nicotinic acetylcholine receptors requires lower concentrations of d-TC than are needed to block SK current in the same cells (Nohmi and Kuba, 1984; Kuba et al., 1989).

Although mesencephalic DA neurons in brain slices exhibit a very regular pattern of spontaneous firing (Shepard and Bunney, 1988), burst firing is commonly seen in these neurons in vivo (Grace and Bunney, 1984a). Shepard and Bunney (1988, 1991) reported that apamin (1 μM) caused the emergence of bursting activity in dopamine neurons of the substantia nigra pars compacta in brain slices that was very similar to bursting seen in vivo. By contrast, Seutin et al.
(1993) found that lower concentrations of apamin alone (100–300 nM) did not change the pattern or rate of spontaneous firing of mesencephalic dopamine neurons studied in horizontal brain slices but increased the incidence of N-methyl-D-aspartate-induced burst activity in these neurons. In the present study, spontaneous firing rate was unchanged and burst firing was only occasionally observed in dopaminergic VTA neurons recorded in coronal brain slices in the presence of apamin (40–100 nM). One possible explanation for these different observations may be that the amount of endogenous glutamate present in the slice may vary according to the way the slice is prepared, and if endogenous glutamate is present, the addition of apamin may elicit bursting similar to that observed after the application of N-methyl-D-aspartate in the presence of apamin to mesencephalic dopamine neurons (Seutin et al., 1993). Alternatively, because different SK isoforms are blocked by different apamin concentrations (Köhler et al., 1996; Ishii et al., 1997), it is possible that lower concentrations of apamin preferentially block SK channels on dopaminergic VTA neurons themselves. Higher concentrations of apamin may additionally block isoforms of SK channels on nondopaminergic neurons present in the slice, which may then release excitatory amino acid transmitters onto dopaminergic neurons, resulting in bursting.

The primary sequences for all of the cloned SK isoforms contain many potential phosphorylation sites, which raises the possibility of modulation by neurotransmitters that activate protein kinases (Köhler et al., 1996). In situ hybridization studies have demonstrated that mRNA for the apamin-insensitive isoform SK1 is found in cell types with apamin-insensitive AHPs (Ishii et al., 1997). The apamin-insensitive \( I_{\text{AHP}} \) has been studied extensively in rat CA1 hippocampal pyramidal cells, where it has been shown to be reduced by norepinephrine, serotonin, and histamine through the adenyl cyclase/cAMP/protein kinase A pathway (Pedarzani and Storm, 1993). This current is also inhibited by acetylcholine and metabotropic glutamate receptor activation but probably through a different signaling pathway (Pedarzani and Storm, 1993). The apamin-sensitive \( I_{\text{AHP}} \) is found in brain areas where mRNAs for the apamin-sensitive isoforms SK2 and SK3 are localized. Muscarine has been shown to reduce the apamin-sensitive \( I_{\text{AHP}} \) in bullfrog sympathetic ganglion cells (Pennefather et al., 1985), but the apamin-sensitive medium-duration AHP in cat sensorimotor cortex is not affected by muscarinic or \( \beta \)-adrenergic agonists (Schwindt et al., 1988a). Interestingly, in human neocortical neurons (predominantly temporal cortex), the apamin-sensitive medium-duration AHP is reduced by serotonin, norepinephrine and muscarine (Lorenzon and Foehring, 1992).

We have shown previously that serotonin potentiates ethanol-induced excitation of dopaminergic VTA neurons (Brodie et al., 1995) through the activation of 5-hydroxytryptamine 2 receptors. 5-Hydroxytryptamine 2 receptors have been shown to be coupled to inositol trisphosphate production (Uneyama et al., 1993). Metabotropic glutamate receptor-coupled inositol trisphosphate production has been shown to inhibit \( I_{\text{AHP}} \) in rat dentate granule neurons (Abdul-Ghani et al., 1996). Further experiments will be necessary to determine whether serotonin potentiation of ethanol excitation in VTA neurons is due to a reduction in SK current. In summary, the present study demonstrates that reduction in SK current by apamin or d-TC potentiates ethanol excitation of dopaminergic VTA neurons, which suggests that the amount of SK current affects the sensitivity of these reward neurons to ethanol excitation. Therapeutic agents that modulate SK current or modify modulation of this current by endogenous neurotransmitters could be of potential benefit in the treatment of alcoholism by modulating the rewarding effect of ethanol on dopaminergic VTA neurons.

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Send reprint requests to: Sarah B. Appel, Ph.D., Department of Physiology and Biophysics (MC 901), University of Illinois at Chicago, College of Medicine, 835 S. Wolcott Ave., Chicago, IL 60612-7342. E-mail: sappel@uic.edu