Electrophysiological Effects of Cocaethylene, Cocaine, and Ethanol on Dopaminergic Neurons of the Ventral Tegmental Area

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ABSTRACT

Coabuse of ethanol and cocaine is one of the most commonly used drug combinations and results in the formation of cocaethylene by the liver. Dopaminergic neurons of the ventral tegmental area (VTA) play a key role in the rewarding properties of drugs of abuse, including ethanol and cocaine. We have previously examined the electrophysiological effects of ethanol and cocaine, and their combined effects on these neurons. The present study investigates the electrophysiological effects of cocaethylene on dopaminergic VTA neurons with extracellular single-unit recording in brain slices from Fischer 344 rats. Cocaethylene (1–10 mM) decreased the firing rate of dopaminergic VTA neurons, similar to the effect of cocaine over this concentration range. This inhibition was blocked by the D2 dopamine receptor antagonist, sulpiride (2 μM). At a lower concentration, cocaethylene (500 nM) potentiated ethanol-induced excitation of these neurons, similar to the effect of cocaine (500 nM) previously reported. This potentiation of ethanol excitation by cocaethylene was reversed by the 5-HT2 antagonist ketanserin (5 μM). These data suggest that cocaethylene acts through a serotonergic mechanism at low concentrations to potentiate ethanol excitation of reward neurons and through a dopaminergic mechanism at high concentrations. The potency of cocaethylene in both of these actions is similar to that of cocaine. These effects of cocaethylene are likely to contribute to the synergistic effect on the dopaminergic reward pathway when ethanol and cocaine are used together; this may help to explain the high incidence of coabuse of ethanol and cocaine.

The coabuse of cocaine and alcohol is one of the most commonly used drug combinations in the U.S.A. In a national survey, over 96% of cocaine users also reported alcohol use over the same month period (concurrent use) and of these over 85% took the two drugs together (simultaneous use) (Grant and Harford, 1990). Simultaneous use of alcohol and cocaine can result in gross impairment of judgment and psychomotor skills, increasing the risk of traffic, occupational and other accidents, overdose, and death (Grant and Harford, 1990).

Coabuse of cocaine and alcohol results in the formation of cocaethylene, a metabolite that is found in urine and blood samples obtained from users of both cocaine and ethanol (Rafia and Epstein, 1979; Hearn et al., 1991). Cocaethylene is formed in the liver by the transesterification of cocaine when ethanol is present (Hearn et al., 1991; Jatlow et al., 1991). Cocaethylene is a pharmacologically active metabolite, and like cocaine, binds to the dopamine transporter and inhibits the reuptake of dopamine, increasing the extracellular concentration of dopamine (Hearn et al., 1991; Jatlow et al., 1991; Woodward et al., 1991; Bradberry et al., 1993). Cocaethylene also binds to the serotonin transporter and can increase the extracellular concentration of serotonin through inhibition of reuptake (Hearn et al., 1991; Bradberry et al., 1993).

Cocaethylene is also behaviorally active and like cocaine has rewarding effects in both humans and animals. Cocaethylene causes euphoria in humans as measured by self-rating scales of the intensity of the “high” (McCance et al., 1995). Self-administration of cocaethylene has been demonstrated in monkeys (Jatlow et al., 1991). In rats, cocaethylene has been shown to be reinforcing in an alley running task (Raven et al., 2000) and to effectively substitute for cocaine in a drug discrimination protocol (Woodward et al., 1991).

The mesolimbic/mesocortical dopamine pathway mediates the rewarding properties of cocaine and ethanol (Wise, 1987). Dopaminergic neurons in the ventral tegmental area (VTA) are the cells of origin of the mesolimbic/mesocortical dopamine pathway and provide dopaminergic innervation of the nucleus accumbens (Oades and Halliday, 1987). Ethanol’s rewarding properties appear to result from its ability to ex-

ABBREVIATIONS: VTA, ventral tegmental area; 5-HT, serotonin; DA, dopamine; aCSF, artificial cerebrospinal fluid; ANOVA, analysis of variance.
cite dopaminergic cell bodies in the VTA (Gessa et al., 1985; Brodie et al., 1990). Cocaine’s rewarding properties involve its blockade of dopamine reuptake in the nucleus accumbens, which increases and prolongs the effect of synaptically released dopamine (Ritz et al., 1987; Koob and Bloom, 1988). Animals will self-administer ethanol directly into the VTA (Gatto et al., 1994; Rodd et al., 1998). By contrast, animals will self-administer cocaine into the nucleus accumbens (McBride et al., 1999), but not the VTA (De La Garza et al., 1998). These data indicate that, although the rewarding effect of both of these drugs are mediated by the mesolimbic pathway, their primary action occurs at different points on the pathway: ethanol at the dopaminergic cell bodies in the VTA and cocaine in the dopamine terminal fields in the nucleus accumbens.

We have previously studied the effects of ethanol, cocaine, and their combined effects on dopaminergic VTA neurons recorded in brain slices. Ethanol increases the firing rate of dopaminergic VTA neurons in a concentration-dependent manner over a behaviorally relevant range of ethanol concentrations (20–200 mM) (Brodie et al., 1990). A low concentration of cocaine (500 nM), had a minimal effect on the baseline firing rate of dopaminergic VTA neurons but potentiated ethanol-induced excitation of these neurons (Bunney et al., 2000a). This potentiation was blocked by the 5-HT₂ antagonist ketanserin, suggesting that this action of cocaine was due to its inhibition of serotonin reuptake. Higher concentrations of cocaine (1–10 μM) caused a concentration-dependent decrease in the firing rate of dopaminergic VTA neurons (Brodie and Dunwiddie, 1990; Bunney et al., 2000a). The cocaine-induced reduction in firing rate was blocked by the D₂ receptor antagonist sulpiride, suggesting that this effect is due to cocaine-induced inhibition of dopamine (DA) reuptake.

To our knowledge, no electrophysiological studies of the effect of cocaethylene on brain neurons have been published. The present study was undertaken to determine the effect of cocaethylene and the combined effects of cocaethylene and ethanol on the firing rate of dopaminergic VTA “reward” neurons. The effects of cocaethylene were also compared with the effects of cocaine on these neurons. Some of the results have been previously reported in abstract form (Bunney et al., 1998, 2000b).

**Materials and Methods**

**Brain Slice Preparation.** Fischer 344 rats (100–200 g) were sacrificed by cervical dislocation; this method of sacrifice is rapid and acceptable for rats of this size. 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. The full methodology for our preparation of brain slices of the VTA has been published previously (Brodie and Dunwiddie, 1990; Bunney et al., 2000a). Briefly, the rat brain was removed rapidly from the cranium and kept chilled and moist during dissection. A tissue block containing the VTA and substantia nigra was mounted in a vibratome and submerged in chilled, oxygenated, artificial cerebrospinal fluid (aCSF). Coronal sections (400 μm thick) were cut, and the tissue was mounted directly in the recording chamber. Equilibration time of 1 h was allowed after placement of tissue in the recording chamber before recordings were made. The slice sat on a mesh platform, totally submerged in the recording chamber, and was weighted down with small platinum logs to increase the stability of recordings. A superfusion system maintained the flow of medium at 2 ml/min; the temperature in the recording chamber was about 35°C. 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 (about 300 μl) used in these studies permitted the rapid application and washout of drug solutions. Composition of the aCSF in these experiments was (in mM): NaCl 126, KCl 2.5, NaH₂PO₄ 1.24, CaCl₂ 2.4, MgSO₄ 1.3, NaHCO₃ 26, d-glucose 11; aCSF is saturated with 95%/5% CO₂ at 35°C (pH 7.4).

**Cell Identification.** We positioned electrodes into the VTA by visual guidance; the VTA is clearly visible in the fresh tissue as a gray area medial to the darker substantia nigra, and separated from the nigra by white matter. Note that dopaminergic neurons have been shown to have electrophysiological characteristics very different from nondopaminergic cells in this region (Grace and Bunney, 1983). Dopamine-containing neurons possess broad (>2.5 ms) action potentials often with an inflection or “notch” on the rising phase, fire spontaneously and regularly at 0.5 to 5 Hz, and show inhibition by dopamine (Bunney et al., 1973; Aghajanian and Bunney, 1977; Grace, 1987). Only neurons meeting these electrophysiological criteria were studied. Only one neuron was used per slice.

**Drug Administration.** Drugs were added to the aCSF 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. Typically, drugs reach equilibrium in the tissue after 2 to 3 min of application. Cocaethylene and (−)-cocaine HCl were obtained from Research Biochemicals International (Natick, MA). Cocaethylene and cocaine effects required up to 1 h of washout to fully reverse; therefore, lower concentrations of cocaethylene or cocaine were always tested (in the absence and presence of ethanol) before higher concentrations of cocaethylene or cocaine were administered. Each concentration of cocaethylene was applied for 20 min before ethanol responses in the presence of cocaine were tested.

A stock solution of 95% ethanol (v/v USP) was used in the pump, and infusion of ethanol never exceeded 1% of the flow rate of the aCSF. Ethanol was administered for 6 to 7 min to ensure that measurements were made after the full ethanol concentration was reached in the tissue and the peak drug effect was attained.

The behaviorally active range for blood ethanol concentrations in the rat extends from 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 (mean lethal dose ~200 mM) (Haggard et al., 1940). Rats will self-administer 40 mM ethanol directly into the VTA, indicating that this concentration is rewarding in the whole animal (Rodd et al., 1998). The present study examined ethanol concentrations in the range of 40 to 120 mM, pharmacologically relevant, sublethal concentrations in the rat.

**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. The Fintronics amplifier used in these recordings includes a window discriminator, the output of which was fed to both a rectilinear pen recorder, and a computer-based data acquisition system that was used for on-line and off-line analysis of the data. The multiplexed output of the Fintronics amplifier was displayed on an analog storage oscilloscope, for accurate adjustment of the window levels used to monitor single units. An IBM-PC-based data acquisition system was used to calculate, display, and store the frequency of firing over 5-s and 1-min intervals. Firing rate was determined before and during drug application. Firing rate was calculated over a 1-min interval immediately prior to drug administration and a 1-min interval during the peak drug effect; drug-induced changes in firing rate were expressed as the percentage of...
change from the control firing rate according to the formula \( \frac{FR_D - FR_C}{FR_C} \times 100 \), where \( FR_D \) is the firing rate during the peak drug effect and \( FR_C \) is the control firing rate. The change in firing rate thus is expressed as a percent of the initial firing rate, which controls for small changes in firing rate that may occur over time.

**Statistical Analysis.** Averaged numerical values were expressed as the mean ± S.E.M. The significance of firing rate changes before and after a single drug concentration was assessed with a paired \( t \) test. For effects of multiple drug concentrations or more than one drug, an appropriate one- or two-way analysis of variance (ANOVA) was used, followed by Student-Newman-Keuls post hoc comparisons when needed. Statistical analyses were performed with SigmaStat (SPSS, Chicago, IL).

### Results

Data in this study were gathered in extracellular single unit recordings from 36 VTA neurons that were identified as dopaminergic according to electrophysiological criteria (see Materials and Methods). All neurons fired spontaneous action potentials, with regular interspike intervals, and their firing rates ranged from 0.71 to 2.8 Hz; the mean firing rate was 1.45 ± 0.09 Hz (S.E.M., \( n = 36 \)).

**Cocaethylene Concentration-Response Curve.** Cocaethylene reduced the spontaneous firing rate of dopaminergic VTA neurons. The cumulative concentration response to increasing concentrations of cocaethylene (1–10 \( \mu \)M) is shown in Fig. 1A. Cocaethylene caused a concentration-dependent reduction in the firing rate of this dopaminergic VTA neuron. The concentration-dependent reduction in firing rate seen with cocaethylene was similar to the concentration-dependent reduction seen with cocaine in the same neuron (Fig. 1B). Note that both the inhibition of firing rate by cocaethylene and cocaine reversed upon washout. Figure 2 illustrates the pooled concentration-response curves for cocaine and cocaethylene (0.5–10 \( \mu \)M) from 11 experiments similar to that shown in Fig. 1. Each neuron was tested with cocaethylene and cocaine, separated by at least 1 h of washout before the second drug was tested. The mean control firing rate prior to testing of cocaethylene and cocaine in these 11 neurons was 1.72 ± 0.18 Hz. The mean percent decrease in firing rate for cocaethylene ranged from −6.0 ± 2.9% (\( n = 7 \)) with 500 nM cocaethylene, to −44.0 ± 6.2% (\( n = 8 \)) with 10 \( \mu \)M cocaethylene. For cocaine, the mean percent decrease in firing rate ranged from −2.8 ± 1.4% (\( n = 7 \)) with 500 nM, to −49.0 ± 8.2% (\( n = 8 \)) with 10 \( \mu \)M. A two-way ANOVA was used to compare the concentration-response curves for cocaethylene and cocaine (Fig. 2). The effects of cocaethylene and cocaine were concentration-dependent (\( F = 19.75, \text{df} = 4, 84, P < 0.001 \)), and there was no significant difference between the effect of cocaethylene and cocaine (\( P > 0.05 \)).

**Sulpiride, a D\(_2\) Antagonist, Blocks the Cocaethylene-Induced Inhibition of Dopaminergic VTA Neurons.** Cocaine and cocaethylene inhibit the reuptake of dopamine, leading to the accumulation of extracellular dopamine. Dopamine can act on D\(_2\) autoreceptors to cause a decrease in firing rate of dopaminergic VTA neurons (White and Wang, 1984). These autoreceptors have been confirmed to be D\(_2\) and not D\(_3\) by the use of knockout mice (Mercuri et al., 1997; Koeltzow et al., 1998). The following experiment tested whether cocaethylene inhibition of dopaminergic VTA neurons could be blocked by the D\(_2\) receptor antagonist sulpiride. Four dopaminergic VTA neurons were tested with increasing concentrations of cocaethylene (0.5, 1, 2, and 5 \( \mu \)M) before and again in the presence of sulpiride (2 \( \mu \)M). Figure 3 shows pooled concentration-response curves for these neurons, in which mean percent change in firing rate is plotted as a function of time; each vertical bar represents the average firing rate over a 5-s interval. Horizontal bars indicate the duration of bath application of the concentration of cocaethylene (A) and cocaine (B) noted above the bar. Responses in A and B were measured in the same dopaminergic VTA neuron. The percent reduction in firing rate produced in this neuron by cocaethylene (A) was −25.5% (1 \( \mu \)M), −26.6% (2 \( \mu \)M), −63.2% (5 \( \mu \)M), and −80.8% (10 \( \mu \)M). The percent reduction in firing rate produced in this neuron by cocaine (B) was −13.5% (1 \( \mu \)M), −19.9% (2 \( \mu \)M), −49.1% (5 \( \mu \)M), and −89.0% (10 \( \mu \)M). Note that both the inhibition induced by cocaethylene and by cocaine reversed upon washout.

![Fig. 1.](image)

Cocaethylene and cocaine reduce the firing rate of dopaminergic VTA neurons in a concentration-dependent manner. Firing rate is plotted as a function of time; each vertical bar represents the average firing rate over a 5-s interval. Horizontal bars indicate the duration of bath application of the concentration of cocaethylene (A) and cocaine (B) noted above the bar. Responses in A and B were measured in the same dopaminergic VTA neuron. The percent reduction in firing rate produced in this neuron by cocaethylene (A) was −25.5% (1 \( \mu \)M), −26.6% (2 \( \mu \)M), −63.2% (5 \( \mu \)M), and −80.8% (10 \( \mu \)M). The percent reduction in firing rate produced in this neuron by cocaine (B) was −13.5% (1 \( \mu \)M), −19.9% (2 \( \mu \)M), −49.1% (5 \( \mu \)M), and −89.0% (10 \( \mu \)M). Note that both the inhibition induced by cocaethylene and by cocaine reversed upon washout.
firing rate of VTA neurons; mean firing rate was 1.06 ± 0.12 before sulpiride and 1.23 ± 0.11 in the presence of 2 μM sulpiride (n = 4; paired t test, P = 0.01).

A Low Concentration of Cocaethylene Enhances Ethanol Excitation of Dopaminergic VTA Neurons. We have previously shown that ethanol increases the firing rate of dopaminergic VTA neurons and that a low concentration of cocaine (500 nM) potentiates the ethanol-induced excitation of these neurons. In the present study, we tested the effect of ethanol in the presence of a low concentration of cocaethylene (500 nM). Application of 500 nM cocaethylene alone to 27 dopaminergic VTA neurons caused only a very small decrease in the mean baseline firing rate from 1.46 ± 0.11 Hz to 1.33 ± 0.11 Hz (paired t test, t = 5.26, df = 26, P < 0.001). Figure 4A shows the response of a dopaminergic VTA neuron to 80 mM ethanol alone, and Fig. 4B shows the response of the same neuron to ethanol (80 mM) in the presence of cocaethylene (500 nM). In the control condition before cocaethylene, this concentration of ethanol increased the firing rate by 21.9%. Note that in this neuron, 500 nM cocaethylene caused a small increase in baseline firing rate. In the presence of 500 nM cocaethylene, the same concentration of ethanol increased the firing of this neuron by 35.7%. Similar experiments were performed testing ethanol (40, 80, and 120 mM) before and in the presence of 500 nM cocaethylene in 12 dopaminergic VTA neurons. Figure 5 shows the pooled results from these experiments. Ethanol caused a concentration-dependent increase in firing rate. Cocaethylene enhanced this ethanol-induced excitation. A two-way ANOVA showed both the cocaethylene enhancement of ethanol excitation (F = 6.26, df = 1.50; P = 0.016) and concentration dependence of the ethanol excitation (F = 4.02, df = 2.50; P = 0.024) were statistically significant. There was no significant interaction between the effect of cocaethylene and ethanol concentration (P > 0.05).

A Higher Concentration of Cocaethylene Enhances Ethanol Excitation of VTA Dopamine Neurons in the Presence of Sulpiride, a D2 Receptor Antagonist. The following experiments were performed to determine whether a higher concentration of cocaethylene (2 μM) would enhance ethanol excitation when D2 receptors were blocked by sulpiride. Excitation by 80 and 120 mM ethanol was measured in each dopaminergic VTA neuron in the control condition, again in the presence of sulpiride (2 μM) and lastly after the subsequent addition of 2 μM cocaethylene with sulpiride still present; pooled data for six dopaminergic VTA neurons are shown in Fig. 6. In the presence of sulpiride, 2 μM cocaethylene enhanced the ethanol excitation. A two-way repeated-measures ANOVA indicated that ethanol excitation was concentration-dependent (F = 19.79; df = 1.5; P < 0.01) and that there was a significant effect of the sulpiride-cocaethylene conditions (F = 9.46; df = 2.10; P = 0.005). Specifically, Student-Newman-Keuls post hoc comparison showed that, in the presence of sulpiride, 2 μM cocaethylene significantly (P < 0.01) enhanced the ethanol responses compared with responses in sulpiride alone. Ethanol responses in sulpiride alone were not significantly different from control (P > 0.05).

Sulpiride alone did not change the spontaneous firing rate of VTA neurons; mean firing rate was 1.32 ± 0.23 before sulpiride and 1.36 ± 0.21 in the presence of 2 μM sulpiride (n = 6; paired t test, P > 0.05). In contrast to the inhibition
in firing rate caused by 2 μM cocaethylene seen in Fig. 1, in the presence of sulpiride, cocaethylene did not significantly decrease the firing rate of VTA neurons, which is consistent with D₂ receptor blockade. The mean firing rate in the presence of sulpiride (2 μM) just before the addition of 2 μM cocaethylene was 1.43 ± 0.27 and was 1.36 ± 0.24 in the presence of 2 μM cocaethylene (n = 6; paired t test, P > 0.05).

**Ketanserin, a 5-HT₄ Antagonist, Reverses Cocaethylene Potentiation of Ethanol Excitation in Dopaminergic VTA Neurons.** Cocaine and cocaethylene inhibit the reuptake of serotonin, leading to the accumulation of extracellular serotonin. We have previously shown that cocaine potentiation of ethanol excitation in dopaminergic VTA neurons is blocked by the 5-HT₄ antagonist ketanserin, suggesting that this action of cocaine is due to its inhibition of serotonin reuptake. The following experiment tested whether cocaethylene potentiation of ethanol excitation in dopaminergic VTA neurons could be blocked by the 5-HT₄ antagonist ketanserin. Each dopaminergic VTA neuron was tested with ethanol (120 mM) in the control condition, again in the presence of cocaethylene (500 nM), and after the subsequent addition of 1 μM ketanserin. The mean firing rate in the presence of cocaethylene (500 nM) was 1.36 ± 0.24 and was 1.29 ± 0.21 in the presence of ketanserin (n = 6; paired t test, P < 0.05).

**Ketanserin reverses cocaethylene potentiation of ethanol excitation.** In the presence of cocaethylene (500 nM) and ethanol (120 mM), the mean firing rate in the presence of ketanserin (1 μM) was 1.29 ± 0.21, which was significantly lower than the firing rate in the presence of cocaethylene (500 nM) alone (1.36 ± 0.24; paired t test, P < 0.05).

**Fig. 4.** Cocaethylene enhances ethanol-induced excitation of a dopaminergic VTA neuron. Firing rate is plotted as a function of time; each vertical bar represents the average firing rate over a 5-s interval. Horizontal bar indicates the duration of bath application of ethanol (80 mM). A, in the control condition before cocaethylene, this concentration of ethanol increased the firing rate by 21.9%. B, in the presence of 500 nM cocaethylene, the same concentration of ethanol (80 mM) increased the firing of this same neuron by 35.7%.

**Fig. 5.** Pooled concentration-response curves show significant potentiation of ethanol-induced excitation by 500 nM cocaethylene. Dopaminergic VTA neurons (n = 12) were tested with ethanol (40, 80, and 120 mM) before and during application of cocaethylene (500 nM). Mean percent change in firing rate induced by ethanol is plotted versus ethanol concentration; error bars indicate S.E.M. The number of neurons tested with ethanol (40, 80, and 120 mM) were 4, 12, and 12, respectively. Cocaethylene significantly enhanced the ethanol-induced increase in firing rate (two-way ANOVA, P = 0.016), and the ethanol excitation was concentration-dependent (P = 0.024).

**Fig. 6.** Pooled concentration-response curves show potentiation of ethanol-induced excitation by 2 μM cocaethylene in the presence of the D₂ antagonist sulpiride. Dopaminergic VTA neurons were tested with ethanol (80 and 120 mM) before and during applications of sulpiride (2 μM), and further tested during applications of cocaethylene (2 μM) in the presence of sulpiride (n = 6). Concentrations of ethanol were used that produced less than a 50% excitation prior to cocaine administration. Two micromolar cocaine significantly increased ethanol-induced excitation in the presence of sulpiride. (Two-way repeated measures ANOVA, P = 0.005; Student-Newman-Keuls, P < 0.01.) Sulpiride alone did not significantly increase ethanol excitation (Student-Newman-Keuls, P > 0.05).
addition of 5 μM ketanserin in the continued presence of cocaethylene. The mean percent increases in firing rate in response to ethanol for the nine neurons tested are shown in Fig. 7. Ethanol alone caused a characteristic increase in firing rate. Cocaethylene (500 nM) significantly enhanced the ethanol-induced excitation (one-way repeated-measures ANOVA, F = 9.16, df = 2,16, P = 0.002; Student-Newman-Keuls post hoc comparison, P = 0.002). In the presence of ketanserin and cocaethylene, the magnitude of the ethanol excitation was significantly reduced compared with its magnitude in the presence of cocaethylene alone (Student-Newman-Keuls post hoc comparison, P = 0.016), but was not significantly different from the magnitude of the ethanol excitation before cocaethylene administration (Student-Newman-Keuls post hoc comparison, P > 0.05).

**Discussion**

To our knowledge, this is the first report of electrophysiological effects of cocaethylene on brain neurons. The present study demonstrates that cocaethylene (1–10 μM) causes a concentration-dependent reduction in the firing rate of dopaminergic VTA neurons. This inhibition was blocked by the D₂ receptor antagonist, sulpiride. Cocaethylene has been shown to bind to the dopamine transporter and to inhibit the reuptake of dopamine, leading to an increase in the extracellular concentration of dopamine (Jatlow et al., 1991; Bradberry et al., 1993). Dopamine has been shown to decrease the firing rate of mesencephalic dopaminergic neurons through an action on D₂ autoreceptors (Lacey et al., 1987). The cocaethylene-induced reduction in firing rate seen in the present study, therefore, is likely to be mediated by cocaethylene inhibition of DA reuptake in the VTA, resulting in increased extracellular DA that acts on inhibitory D₂ autoreceptors. This is similar to the mechanism underlying cocaine inhibition of dopaminergic VTA neurons previously reported (Brodie and Dunwiddie, 1990; Lacey et al., 1990). Inhibitory feedback, to the VTA, from the nucleus accumbens has been shown, in vivo, to increase the inhibitory action of cocaine on dopaminergic VTA neurons and may represent an additional mechanism by which cocaine inhibits these neurons (Einhorn et al., 1988).

The potency of cocaethylene to inhibit the firing of dopaminergic VTA neurons was found to be equal to that of cocaine in the present study. The similarity in the concentration-response curves for cocaine and cocaethylene may be explained by their similar affinity for the dopamine transporter. Cocaethylene and cocaine have been shown to be equipotent at inhibiting [3H]mazindol and [3H]GR 12395 binding to the dopamine transporter (Hearn et al., 1991; Jatlow et al., 1991). Cocaethylene and cocaine also show equal potency in inhibiting dopamine uptake into synaptosomes, and in increasing the extracellular dopamine concentration in the nucleus accumbens, as measured by microdialysis after intravenous injection of both substances (Jatlow et al., 1991; Bradberry et al., 1993). The concentration-response curves for cocaethylene and cocaine inhibition of dopaminergic VTA neurons in brain slices from Fischer 344 rats measured in the present study are similar to the concentration-response curve for cocaine previously reported for dopaminergic VTA neurons in brain slices from Sprague-Dawley rats (Brodie and Dunwiddie, 1990; Lacey et al., 1990).

In the present study, we found that a low concentration of cocaethylene (500 nM) enhanced ethanol-induced excitation of dopaminergic VTA neurons. This effect of cocaethylene is similar to the enhancement of ethanol excitation of these neurons by 500 nM cocaine, which we have recently reported (Bunney et al., 2000a). Both the cocaethylene enhancement of ethanol excitation observed in the present study and the cocaine enhancement of ethanol excitation in our previous study were reversed by the 5-HT₂ antagonist ketanserin. Prior to the studies with cocaethylene and cocaine, we demonstrated that ethanol excitation of these neurons was potentiated by serotonin, the 5-HT₂ agonists (±)-2,5-dimethoxy-4-iodoamphetamine and α-methylserotonin (Brodie et al., 1995), and by the monoamine reuptake inhibitor clomipramine (Trifunovic and Brodie, 1996). These data support the idea that the potentiation of ethanol-induced excitation of dopaminergic VTA neurons by low concentrations of cocaethylene and cocaine is due to inhibition of serotonin reuptake in the VTA, leading to an increase in extracellular serotonin, which then acts on 5-HT₂ receptors.

Cocaethylene appears to have a lower affinity than cocaine for the serotonin transporter in some brain areas. For example, cocaethylene was 40-fold less potent than cocaine in inhibiting [3H]paroxetine binding to the serotonin transporter in postmortem human frontal cortex (Hearn et al., 1991). Cocaethylene was 7-fold less potent than cocaine in inhibiting serotonin reuptake in striatal or cortical synaptosomes (Bradberry et al., 1993). Cocaethylene also caused less

![Fig. 7. Ketanserin reverses the potentiation of ethanol (ETOH)-induced excitation produced by 500 nM cocaethylene (Cocaeth). Dopaminergic VTA neurons (n = 9) were tested with ethanol (120 mM) before and during application of cocaethylene (500 nM), and again during the application of the 5-HT₂ antagonist ketanserin (5 μM) in the continued presence of cocaethylene. Cocaethylene (500 nM) significantly increased the ethanol-induced excitation (*one-way repeated measures ANOVA, P = 0.002; Student-Newman-Keuls post hoc comparison, P = 0.002). In the presence of ketanserin and cocaethylene, the magnitude of the ethanol excitation was significantly reduced, compared with its magnitude in the presence of cocaethylene alone (Student-Newman-Keuls post hoc comparison, P = 0.016) but was not significantly different from the magnitude of the ethanol excitation before cocaethylene administration (Student-Newman-Keuls post hoc comparison, P > 0.05).](image-url)
accumulation of serotonin in the striatum than cocaine, as measured with microdialysis (Bradberry et al., 1993). In contrast, we saw a significant potentiation of the ethanol-induced excitation in dopaminergic VTA neurons with the same (500 nM) concentration of cocaethylene (present study) and cocaine (Bunney et al., 2000a). Because this effect appears to be mediated by inhibition of serotonin reuptake, these data suggest that cocaethylene and cocaine may inhibit serotonin reuptake in the VTA with similar potency.

At higher concentrations of cocaethylene (1–10 μM), dopaminergic VTA neurons were inhibited (Figs. 1 and 2). This is likely to be due to an inhibition of dopamine reuptake by the higher concentrations of cocaethylene. The increased dopamine, acting on D₂ receptors, causes inhibition in the firing rate of these neurons (White and Wang, 1984). We hypothesized that if a D₂ receptor antagonist (sulpiride) was added to the cocaethylene and ethanol combination, then a higher concentration of cocaethylene (2 μM) might be effective in producing enhancement of ethanol excitation in VTA dopamine neurons. Indeed, we found that, in the presence of 2 μM sulpiride, 2 μM cocaethylene significantly enhanced ethanol excitation (Fig. 6). This further supports the idea that potentiation of ethanol excitation by cocaethylene is mediated by a serotonergic, not a dopaminergic, mechanism.

The low concentration of cocaethylene (500 nM) used in the present study appears to be pharmacologically relevant to cocaine/ethanol coabuse in humans. For example, intranasal administration of 0.95 mg/kg of cocaethylene to human subjects (coabusers of cocaine and ethanol) produced a mean plasma cocaethylene concentration of about 520 nM at 15 min and about 709 nM at 30 min, which resulted in euphoria equivalent to that produced by an equimolar intranasal dose of cocaine (McCance et al., 1995). This dose of cocaine (0.92 mg/kg) resulted in a mean plasma cocaine concentration at 15 min of about 320 nM at the time of peak “high”. In another study, intranasal administration of 96 mg cocaine produced a mean plasma cocaine concentration of 570 nM at time of peak “high” (Javaid et al., 1978). In patients admitted to the hospital who had a measurable cocaethylene level, the mean plasma cocaethylene concentration was 353 nM; in these same patients, the mean plasma cocaine concentration was 386 nM and the mean plasma ethanol concentration was 36.5 mM (Bailey, 1996). These data indicate that the 500 nM concentrations of cocaethylene and cocaine used in the present study produce euphoria in humans and are similar to levels found in the blood of cocaine/ethanol coabusers.

Coabuse of ethanol and cocaine may result in a number of effects on the mesolimbic reward pathway. Our recent work indicates that ethanol directly excites the cell bodies of dopaminergic neurons in the VTA (Brodie et al., 1999), which results in increased DA release in their terminal fields in the nucleus accumbens (Di Chiara and Imperato, 1988; Weiss et al., 1993). Cocaine inhibits the reuptake of DA, thereby increasing the amount of DA accumulating at synapses in the nucleus accumbens (Bradberry and Roth, 1989). These effects of ethanol and cocaine should act synergistically to increase the activity in the mesolimbic DA reward pathway. Furthermore, cocaethylene, a metabolite formed by transetherification of cocaine in the presence of ethanol, also inhibits the reuptake of DA thereby increasing the amount of DA accumulating at synapses in the nucleus accumbens (Jatlow et al., 1991; Bradberry et al., 1993).

The present study demonstrates that a low concentration of cocaethylene, like cocaine (Bunney et al., 2000a), also enhances ethanol-induced excitation of dopaminergic VTA neurons. This potentiation of ethanol excitation by cocaethylene and cocaine would also add to the rewarding effects when cocaine and ethanol are coabused. In summary, it is likely that cocaethylene exerts important actions in two brain areas: in the VTA to enhance ethanol excitation of dopaminergic neurons, and in the nucleus accumbens, to which the dopaminergic VTA neurons project, to block the reuptake of DA by acting at the DA transporter. Both of these actions would serve to increase the extracellular DA concentration in the nucleus accumbens, which should increase the resultant rewarding effect. The fact that cocaethylene produces rewarding effects similar to cocaine but has a much longer half-life may help to explain why use of ethanol with cocaine prolongs the “high” and helps prevent the “crash”, as reported by coabusers (McCance-Katz et al., 1993) and why the coabuse of cocaine and ethanol is so prevalent.

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References


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