COMBINED EFFECTS OF SYSTEMIC ALCOHOL AND NICOTINE ON DOPAMINE RELEASE IN THE NUCLEUS ACCUMBENS SHELL

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(Received 12 February 2007; first review notified 17 April 2007; in revised form 10 May 2007; accepted 29 May 2007; advance access publication 8 August 2007)

Abstract — Aims: This study was undertaken to determine whether simultaneous administration of both alcohol and nicotine systemically would result in an additive dopamine release in the nucleus accumbens (NACC). Moreover, to also investigate whether nicotinic receptors may be mediating these effects of alcohol and nicotine, the effects of mecamylamine, a nicotinic receptor antagonist was also evaluated. Methods: Microdialysis was applied to measure the dopamine overflow in the shell region of NACC. All drugs were administered intraperitoneally. The doses of alcohol ranged from 0.5–2.0 g/kg, and nicotine and mecamylamine 0.25–1.0 mg/kg. Results: An additive effect of combined alcohol and nicotine on dopamine release was obtained. This effect of alcohol and nicotine was dose-dependently blocked by mecamylamine pre-treatment. Conclusions: These findings further support the hypothesis that an additive effect of alcohol and nicotine on the mesolimbic 'reward pathway' may contribute to the high incidence of smoking in alcoholics. Furthermore, nicotinic antagonists can block such effects of combined alcohol and nicotine.

INTRODUCTION

The incidence of smoking is very high among alcoholics. Indeed, higher alcohol consumption is associated with a higher smoking rate (Meyerhoff et al., 2006). This co-morbid condition can have dramatic health consequences, particularly in manifestations of the cancers of the head, neck and oesophagus, as well as duodenal ulcer (Olsen et al., 1985; Johnson and Jennison, 1992; Castellsague et al., 1999; Ko and Cho, 2000). Several factors including genetic predisposition, as well as pharmacokinetic interactions, may be responsible for the tendency to combine alcohol with nicotine (Collins et al., 1996, 1997; True et al., 1999; Howard et al., 2003; Lê et al., 2006; Parnell et al., 2006; Volk et al., 2007; Connor et al., 2007). Another strong contributor may be the rewarding or addictive property of each drug. Thus, a drinker may reach for a cigarette if a synergistic or an additive 'reward' is obtained when the two drugs are combined.

In animal studies, the rewarding effect of a drug may be assessed by the release of dopamine in the nucleus accumbens shell, which receives heavy dopaminergic input from the ventral tegmental area. Together, these two areas comprise an important component of the mesolimbic 'reward pathway' (Koob and Bloom, 1988; Wise and Rompré, 1989; Di Chiara, 1999). Although the effects of alcohol and nicotine individually on this pathway have been extensively studied, very few studies have looked at the combined effects of these drugs. Previously, we had observed that combining an acute dose of alcohol with a central injection of nicotine into the ventral tegmental area (VTA) would result in an additive release of dopamine in the nucleus accumbens shell (Tizabi et al., 2002). Moreover, administration of the nicotinic antagonist, mecamylamine into the VTA blocked alcohol-induced dopamine release. Since nicotine may have varied sites of actions in the brain as well as in the periphery due to extensive distribution of various nicotinic receptors, it was of relevance to determine whether combining systemic administration of both alcohol and nicotine would also result in an additive dopamine release. Furthermore, it was of interest to evaluate the effect of systemic mecamylamine on dopamine release induced by the combination of alcohol and nicotine. Our results indicate an additive effect of systemic alcohol and nicotine on dopamine release which can be blocked by mecamylamine pre-treatment.

MATERIALS AND METHODS

Adult male Wistar rats (280–300 g) were purchased from Harlan Laboratories (Dublin, VA), and were kept in a controlled environment on a 12–h light/dark cycle (on/off at 7:00 AM/7:00 PM), and received food and water ad libitum. All experiments were carried out in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH publication No. 80–23) revised 1996. Ethanol was dissolved in saline (15% w/v) and administered intraperitoneally (i.p.). Nicotine bitartrate and mecamylamine HCl were purchased from RBI (Natick, MA), also dissolved in saline and injected i.p.

An established microdialysis procedure (Yoshimoto and McBride, 1992; Campbell and McBride, 1995; Tizabi et al., 2002) was employed to collect the dialysate in the shell region of nucleus accumbens (NACC) in freely moving animals. Brieﬂy, rats were anesthetized with pentobarbital (60 mg/kg, i.p.) and microdialysis probes (CMA, North Chelmsford, MA) were stereotaxically implanted in the shell region of the NACC and secured to the skull. The NACC shell probe was implanted at a 10 degree angle from the vertical using the following coordinates (in mm) from Bregma: AP +1.4, L 2.4, and D/V −8.4. The animals were allowed at least 48 h to recover from surgery before initiating experiments. Artificial CSF (composition in mM: 145 NaCl, 2.7 KCl, 1.0 MgCl2, 1.2 CaCl2, pH 7.4 with NaH2PO4) was filtered through a 0.2 µm sterile filter and perfused through the probe at 1.0 µl/min for...
60–90 min before baseline samples were collected. Baseline samples were collected every 20 min for 60 min. Alcohol dose ranged from 0.5 to 2.0 g/kg as in a previous study (Tizabi et al., 2002). Nicotine (0.25–1.0 mg/kg) and mecamylamine (0.5–2.0 mg/kg) were injected i.p. in a volume of 1 ml/kg. Mecamylamine was administered 10 min prior to alcohol followed immediately by nicotine. Dialysates were collected every 20 min up to 4 h following treatments. Samples were collected in vials containing 2 µl 0.2 N HCl, frozen on dry ice, and were stored at −80 °C until analysis.

At the end of the experiment, a 1% solution of bromphenol blue was perfused through the probes to verify placements. Only data from animals with verified placements were analyzed.

Measurements of DA in dialysates were carried out using HPLC-EC. The system was fitted with a Rhodiney 8125 micro-injector and a 5 µl loop. The columns used were SepStik Unijet C18 microbore columns, 5 µm, 100 × 1 mm i.d. and 150 × 1 mm i.d. (BAS). The mobile phase was 0.15 M monochloroacetic acid pH 3.0 with 1.0 mM EDTA, 0.86 mM sodium octyl sulfate, 3.5% acetonitrile and 1.8% tetrahydrofuran. The flow rate was 0.2 ml/min. The LC-4C electrochemical detector (BAS) was equipped with a dual glassy carbon electrode at a potential of 800 mV and a sensitivity setting of 0.5 nA/V.

Due to subject variability in the levels of extracellular DA, data for the microdialysis samples were normalized and expressed as a percentage of the baseline. Baseline (100%) was determined as the average of the last three pre-treatment values. Mean percentages were calculated for samples collected at 20 min intervals. Normalized data was analyzed by one- and two-way analysis of variance (ANOVA) with repeated measures, followed by Newman–Keuls post hoc test. The significance level was set, a priori, at \( P < 0.05 \).

**RESULTS**

**Effects of ethanol, nicotine and their combination**

Figure 1 depicts the time course effects of various systemic doses of alcohol, nicotine and their combination on DA release in the shell region of NACC. The basal dopamine level in the dialysate was 2.0 ± 0.3 nM (\( N = 25 \)). Alcohol, nicotine and their combination, dose-dependently increased dialysate DA. The lowest dose of alcohol (0.5 g/kg) resulted in significant (\( P < 0.05 \)) increase in dopamine level in the dialysate at 20 and 40 min. At higher doses, the effect was higher and more sustained [F(2, 23) = 6.1, \( P < 0.02 \)]. Nicotine at the lowest dose (0.25 mg/kg) also resulted in significant (\( P < 0.05 \)) increase in dopamine level in the dialysate at 20 and 40 min. At higher doses, effects were higher and more sustained [F(2, 23) = 5.6, \( P < 0.02 \)]. The combination treatment resulted in higher and more sustained release of DA compared to either drug alone as these effects reached as high as 73 ± 9% over the baseline, and lasted for as long as 1 h after the treatment [F(2, 23) = 9.5, \( P < 0.001 \)].

**Effects of mecamylamine pretreatment**

Figure 2 depicts the time course of effects of various doses of mecamylamine pre-treatment on dopamine release in the NACC shell following a combination dose of alcohol and nicotine. The combination dose chosen was to reflect moderate doses of alcohol and nicotine, which would result in adequate dopamine release without possible ceiling effect. The basal dopamine level in the dialysate was 2.5 ± 0.4 nM (\( N = 26 \)). Mecamylamine, dose-dependently inhibited the effects of alcohol–nicotine combination [F(2, 23) = 10.2, \( P < 0.001 \)]. Maximal inhibition was already achieved by 1 mg/kg dose. Mecamylamine by itself, at any of the doses used, did not have any effect on DA release (data not shown).
EFFECTS OF SYSTEMIC ALCOHOL AND NICOTINE IN THE NUCLEUS ACCUMBENS SHELL

DISCUSSION

The results of this study indicate that systemic administration of combined alcohol and nicotine in rats, results in a higher release of dopamine from the nucleus accumbens shell compared to each drug alone. Moreover, the effects of such a combination on dopamine release can be blocked by pre-treatment with mecamylamine. These findings are consistent with our previous observation where a combination of systemic alcohol with central administration of nicotine into the VTA resulted in additive or exaggerated dopamine release in the nucleus accumbens shell (Tizabi et al., 2002). Since dopamine release in the NACC shell may reflect the "rewarding" effect of the drug, these findings provide further support for the hypothesis that an increase in pleasurable feeling may contribute to the co-abuse of alcohol and nicotine.

Although the exact mechanism for the enhanced effects of the combined administration of alcohol and nicotine on NACC dopamine release is not known, it is very likely that multiple neurotransmitter receptors contribute to this phenomenon. It is known that a variety of receptors including nicotinic receptors may be mediating the actions of alcohol (Tizabi et al., 2002; Meyerhoff et al., 2006). Similarly, the actions of nicotine in the mesolimbic pathway may involve subtypes of nicotinic receptors at both the origin and the terminal region (Tizabi et al., 2002). Thus, stimulation of various receptors by alcohol and nicotine could yield higher dopamine overflow compared to each drug alone. It is also possible that one drug may enhance the central sensitivity to the other drug, and hence, a higher dopamine yield may ensue as a result of the combination treatment.

Interactions between alcohol and nicotine are also evident in a variety of in-vitro and in-vivo studies where some of the toxic or adverse effects of alcohol may be prevented by nicotine pre-treatment (Dar et al., 1994; Prendergast et al., 2000; Penland et al., 2003; Tizabi et al., 2003, 2004, 2005). Al-Rejaie and Dar, 2006a,b). Ethanol, on the other hand, may reduce nicotine-induced seizures (Korkosz et al., 2006). Ethanol may also increase the effects of nicotine in an operant behaviour (Popke et al., 2000) or enhance nicotine-induced place preference (Korkosz et al., 2006). Some recent reports indicate a synergistic antinociceptive effect by a combination of alcohol and nicotine (Campbell et al., 2006, 2007). Thus, a number of factors, including genetic predisposition may be responsible for the high incidence of a drinking-smoking combination.

Our findings with mecamylamine, a non-selective nicotinic receptor antagonist, provide additional evidence in support of the postulation that the reinforcing effects of alcohol are at least partially mediated by central nicotinic receptors. It is particularly noteworthy that administration of mecamylamine systemically (Blomqvist et al., 1996; Lé et al., 2000) or into the VTA (Ericson et al., 1998) markedly reduced ethanol intake and preference in rats. Two more recent reports implicate the alpha-6 and particularly the alpha-3 containing a subunit of the nicotinic receptor as a major modulator of dopamine-enhancing effects of ethanol (Larsson et al., 2004; Jerlhag et al., 2006). Since a nicotinic antagonist may also be of therapeutic potential in smoking cessation paradigms (Rose, 2006), it would be of significant clinical relevance to investigate the effects of selective nicotinic antagonists on the simultaneous intake of alcohol and nicotine and on dopamine release in similar paradigms.

In summary, combined effects of alcohol and nicotine on the reward pathway may be a contributing factor to the high incidence of cigarette smoking in alcoholics. Moreover, administration of selective nicotinic antagonists might be of therapeutic potential in reducing the rewarding effects of alcohol and nicotine.

Acknowledgements — Supported by NIAAA P20 (AA104643); NIH/NIMH (2SO6 GM08016-36)

REFERENCES


