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EFFECTS OF INTRAVENOUS COCAINE AND CIGARETTE SMOKING

ON LUTEINIZING HORMONE, TESTOSTERONE AND PROLACTIN IN MEN*

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Abbreviations

LH   luteinizing hormone

PRL   prolactin

T   testosterone

$t_{1/2}$ half-life

Tmax   time to peak plasma concentration

Cmax   peak plasma concentration

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ABSTRACT

Cocaine and nicotine have a number of similar behavioral and neurobiological effects. This study compared the acute effects of cocaine and cigarette smoking on LH, T and prolactin. Twenty-four men who met DSM-IV criteria for cocaine abuse or nicotine dependence were given intravenous cocaine (0.4 mg/kg) or placebo-cocaine, or smoked a low or high nicotine cigarette under controlled conditions. Placebo-cocaine or low nicotine cigarette smoking did not change LH, T or prolactin. Peak plasma levels of 254 ± 18 ng cocaine/ml and 22.6 ± 3.4 ng nicotine/ml were measured at 8 and 14 min, respectively. LH increased significantly after both i.v. cocaine and high nicotine cigarette smoking (P < 0.01). These LH increases were significantly correlated with increases in cocaine and nicotine plasma levels (P < .001-.003), and high nicotine cigarette smoking stimulated significantly greater increases in LH release than i.v. cocaine (P < 0.05). Testosterone levels did not change significantly after either cocaine or after high nicotine cigarette smoking. After i.v. cocaine, prolactin decreased significantly and remained below baseline levels throughout the sampling period (P < 0.05-0.0l). After high nicotine cigarette smoking, prolactin increased to hyperprolactinemic levels within 6 min and remained significantly above baseline levels for 42 min (P < 0.05-0.03). The rapid increases in LH and reports of subjective "high" after both i.v. cocaine and high nicotine cigarette smoking illustrate the similarities between these drugs, and suggest a possible contribution of LH to their abuse-related effects.

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Cocaine abuse and cigarette smoking are major public health problems. Morbidity and mortality associated with cocaine abuse has been difficult to quantify (NIDA, 2002), but cigarette smoking is estimated to account for approximately 430,000 deaths each year from lung cancer, chronic obstructive pulmonary disease and ischemic heart disease (CDC, 1993, 2001). There appear to be a number of parallels between the abuse-related effects of cocaine and nicotine. For example, recent clinical studies have shown that the positive subjective and physiological effects of i.v. nicotine are very similar to the effects of i.v. cocaine in cocaine abusers who smoke cigarettes (Jones et al., 1999). Nicotine and cocaine also have similar pharmacokinetic profiles after both inhalation and intravenous administration (Evans et al., 1996; Rose et al., 1999). The rapid distribution of both nicotine and cocaine, combined with the relatively brief duration of positive subjective effects, is characteristic of drugs with high abuse liability (Balster and Schuster, 1973). Like cocaine abuse, cigarette smoking is very difficult to treat successfully, and relapse rates are high (Jaffe, 1990; Mendelson and Mello, 1996).

There is a general consensus that cigarette smoking, like cocaine abuse, is an addictive disorder (Jaffe, 1990; APA, 1994; CDC, 2001). Both clinical and preclinical studies have shown that i.v. nicotine is self-administered (Henningfield and Goldberg, 1983; Rose and Corrigall, 1997) and there is evidence that nicotine can induce tolerance and physical dependence (Jaffe, 1990; Henningfield et al., 1995). Nicotine, like cocaine, activates the mesolimbic dopamine system, and it has been postulated that the resulting increases in

extracellular dopamine levels may mediate the abuse-related effects of both drugs (Kuhar et al., 1991; Corrigall et al., 1992; Di Chiara, 2000; Watkins et al., 2000). Interestingly, microdialysis studies suggest that administration of equipotent doses of nicotine and cocaine, given in combination, produce additive effects on nucleus accumbens dopamine release (Sziraki et al., 1999; Gerasimov et al., 2000). However, cocaine and nicotine appear to increase extracellular dopamine levels by different mechanisms. Nicotine stimulates dopamine release in the nucleus accumbens by stimulating nicotinic acetylcholine receptors on the cell bodies of mesolimbic dopamine neurons (Watkins et al., 2000), whereas cocaine increases extracellular dopamine levels by blocking dopamine reuptake by the dopamine transporter (Kuhar et al., 1991).

In addition to dopaminergic effects, cocaine also stimulates release of some anterior pituitary, gonadal and adrenal hormones (see for review Mello and Mendelson, 2002). Preclinical studies suggest that these rapid hormonal changes may contribute to the abuse-related effects of cocaine under some conditions (Goeders, 1997; Mello and Mendelson, 2002). In comparison to cocaine, less is known about the acute effects of cigarette smoking on anterior pituitary and gonadal hormones, and the temporal relation of smoking-induced hormonal changes to reports of subjective "high" and "craving". Accordingly, the present study was designed to determine if cigarette smoking and cocaine have similar or different effects on luteinizing hormone (LH), testosterone (T) and prolactin (PRL) and subjective effects measures. In recent clinical studies, cocaine (0.2 and 0.4 mg/kg, i.v.) produced a rapid dose-dependent increase in

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LH levels (Mendelson et al., 2001). Because stimulation of LH release is a robust effect of cocaine administration in humans and in non-human primates, (for review see Mello and Mendelson, 2002), we compared the effects of i.v. cocaine vs placebo-cocaine and high vs low dose nicotine cigarette smoking on LH and T in men who met DSM-IV criteria for cocaine abuse or nicotine dependence. Although gonadal steroid modulation of gonadotropin release through negative feedback mechanisms is well established (Yen et al., 1999), we are unaware of any studies of the interactions between LH and T after acute cocaine administration and cigarette smoking.

The acute effects of cocaine and cigarette smoking on prolactin release were also studied. Prolactin is under inhibitory dopaminergic control and administration of cocaine or exogenous dopamine significantly decreases prolactin in preclinical studies (Mello et al., 1994; for review see Ben-Jonathan and Hnasko, 2001; Mello and Mendelson, 2002). To the extent that nicotine stimulates dopamine release, it might be predicted that cigarette smoking, like cocaine, would decrease prolactin levels after acute administration. Chronic smoking has been associated with low prolactin levels (Fuxe et al., 1989; CDC, 2001), but increases in prolactin levels have been reported after cigarette smoking (Wilkins et al., 1982; Seyler et al., 1986; Kirschbaum et al., 1994) and i.v. nicotine infusion (Newhouse et al., 1990). In rats, acute nicotine administration produces dose-dependent increases in prolactin (Sharp and Beyer, 1986; Fuxe et al., 1989; Hulihan-Giblin et al., 1990).

We now report that i.v. cocaine and high nicotine cigarette smoking each stimulated significant increases in LH release that were temporally correlated with increases in plasma levels of cocaine and nicotine. Moreover, high nicotine cigarette smoking produced significantly greater stimulation of LH than i.v. cocaine. Testosterone levels did not change significantly after any treatment. Cocaine and high nicotine cigarette smoking had opposite effects on prolactin. After cocaine, prolactin decreased significantly, whereas after high nicotine cigarette smoking, rapid and sustained increases in prolactin were measured.

METHODS

Subjects

Twenty-four men were recruited through newspaper advertisements and provided written informed consent for participation in this study. The study was approved by the Institutional Review Board of the McLean Hospital. Twelve men fulfilled American Psychiatric Association Diagnostic and Statistical Manual (DSM-IV) criteria for a diagnosis of current cocaine abuse (305.6) and 12 men fulfilled DSM-IV criteria for current nicotine dependence (305.1). Volunteers with any lifetime DSM-IV Axis 1 disorder other than cocaine abuse and nicotine dependence were excluded. Men who were seeking treatment for cocaine abuse or nicotine dependence, or who were wearing a nicotine patch were excluded. All men selected for this study were in good physical health and had normal medical and laboratory screening examinations. The characteristics of subjects in the cocaine and nicotine groups are summarized in **Table 1**. These subjects did not differ significantly with respect to age and body mass index (BMI). As indicated in **Table 1**, 12 men were assigned to either cocaine or placebo-cocaine conditions and 12 men were assigned to smoking either high nicotine cigarettes or low nicotine cigarettes.

Subjects were admitted to the clinical research ward on the morning of the study day. Subjects were asked to provide a urine specimen for drug testing and levels of breath carbon monoxide and breath alcohol were measured. If subjects fulfilled the drug abstinence criteria described below, the study procedures were explained and any questions or concerns were discussed. After the study was completed, subjects remained on the clinical research ward for two or more hours. Lunch was provided and vital signs were measured at 30 min intervals. When subjects were medically stable and comfortable, they were discharged and

transportation was provided. Subjects were paid for their participation in the

study, consistent with NIH regulations.

Table 1 about here

Drug Abstinence Requirements

It is important for subject safety, as well as to avoid confounding of the dependent variables, to ensure that subjects have not used any drugs before administration of intravenous cocaine. On the morning of each study day, urines were collected and analyzed with a Triage® screen. The Triage® Panel for Drugs of Abuse (Biosite Diagnostics, San Diego, CA) is a rapid multiple immunoassay system for the qualitative detection of the major metabolites of these drugs of abuse in urine at the following cut-off concentrations as recommended by the Substance Abuse and Mental Health Services Administration: Phencyclidine 25 ng/mL, Benzodiazepines 300 ng/mL, Benzoylecgonine, a metabolite of Cocaine, 300 ng/mL, Amphetamines 1000 ng/mL, Tetrahydrocannabinol 50 ng/mL, Opiates 300 ng/mL and Barbiturates 300 ng/mL.

Smoking Abstinence Requirements

Cigarette smokers were asked to abstain from cigarettes and caffeinated beverages after midnight on the night before the study. Carbon monoxide (CO) levels were measured with a Vitalograph Breath CO Monitor (Vitalograph, Inc., Lenexa, Kansas 66215) to assess compliance with smoking abstinence requirements. Cigarette smokers with a CO level above 10 ppm were not allowed to participate and were rescheduled. Cigarette smokers were also tested with the Triage® screen on the study day. Cocaine abusers also were not allowed to smoke or drink caffeinated beverages before the study.

Cocaine and Nicotine Dose Selection

Cocaine: A 0.4 mg/kg, i.v. dose of cocaine was selected on the basis of our previous clinical studies where it usually produced peak plasma cocaine levels over 200 ng/ml. This dose of cocaine has proved to be safe and induced

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significant changes in mood states and physiological responses in our previous clinical studies (Mendelson et al., 2001).

Nicotine: A commercially available, high yield nicotine cigarette (2.3 mg) and a low nicotine cigarette were studied. The high yield nicotine cigarette (Marlboro Red, Phillip Morris brand) contained 15.48 mg of nicotine and 16 mg of tar based on analysis by the Massachusetts Department of Public Health (MDPH, 1998). According to the Massachusetts DPH classification, cigarettes with a nicotine yield of 1.2 mg or higher are high nicotine cigarettes. In preliminary studies, smoking a Marlboro Red cigarette under the conditions described below produced peak plasma nicotine levels above 20 ng/ml. The low nicotine cigarettes contained 1.1 mg of nicotine and delivered 0.1 mg of nicotine and 2.8 mg of tar based on analyses provided by the manufacturer. Low nicotine cigarettes were acquired from Murty Pharmaceuticals Inc. (Lexington, KY 40509).

Cocaine and Nicotine Administration Procedures

These studies were carried out on a clinical research ward. Cocaine or placebo-cocaine was administered intravenously into the antecubital vein of one arm over an interval of one minute. Cigarettes were administered using a controlled smoking procedure designed to standardize puff volume and duration of inhalation (Griffiths et al., 1982). Every 30 sec, subjects were asked to take a puff from a cigarette and hold the smoke for 5 sec. At the end of 5 sec, subjects were asked to exhale. Subjects took 24 puffs over a 12 min smoking period with an inter-puff interval of 25 sec. This is equivalent to approximately two cigarettes. A new lighted cigarette was presented after every four puffs so that changes in cigarette length would not influence puff duration (Nemeth-Coslett and Griffiths, 1984a, 1984b).

Subjects were studied in a semi-supine position, and heart rate, blood pressure and electrocardiograms (EKG) were continuously monitored with a Hewlett-Packard EKG monitor (Model 78 352A) for ten min prior to intravenous cocaine administration or cigarette smoking and for two hours following cocaine or cigarette administration. A physician certified in cardiopulmonary resuscitation was present during each study, and a cardiac defibrillator and appropriate emergency treatment medications were located in the study room.

Sample Collection Procedures

Baseline samples for analysis of LH, prolactin, testosterone and plasma drug levels were collected 10 min before i.v. cocaine or placebo-cocaine injection or the onset of cigarette smoking. Samples for analysis of LH, prolactin, testosterone and plasma cocaine or plasma nicotine were collected at 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 25, 30, 40, 50, 60, 80, and 120 min following completion of the cocaine injection or the onset of smoking a high nicotine cigarette. This rapid sampling frequency was based on previous observations that cocaine levels in plasma increase rapidly within two min after intravenous administration and reach peak levels within 4 to 5 min (Evans et al., 1996; Mendelson et al., 1999, 2001, 2002). Samples for analysis of plasma cocaine levels were collected at the same frequency after placebo-cocaine administration. Samples were collected before and at 6, 12, 20, 30, 60 and 120 min after smoking a low nicotine placebo cigarette. All blood samples for hormone and cocaine analysis were collected from an intravenous catheter placed in the antecubital vein of the arm opposite the arm used for i.v. cocaine injection. The catheter site and collection tubes were covered during the smoking studies so that smoke in the air would not contaminate plasma samples.

Blood samples for hormone analysis and for nicotine analysis were collected in vacutainer tubes without preservative. Blood samples for cocaine

analysis were transferred to heparinized Vacutainer tubes containing sodium fluoride and acetic acid (to prevent the hydrolysis of cocaine). All samples were iced immediately, centrifuged and plasma was removed and frozen at -70° C for cocaine analysis.

Subjective Effects Measures

Subjects were asked to rate how "high" they felt, how much they "craved" cigarettes or cocaine, and how "sick" they felt on a Visual Analogue Scale (VAS) that ranged from zero to 100. Subjects were asked to provide ratings of "high", "craving" and "sick" before and every two minutes for the first 20 min after smoking or cocaine administration, then at less frequent intervals over the next 100 min.

Cocaine Hydrochloride Preparation

Cocaine hydrochloride was acquired from the National Institute of Drug Abuse in powder form and was dissolved in sterile water for intravenous injection by the McLean Hospital pharmacy. Sterility was ensured by passing the solution through a 0.22 micron millipore filter and subjecting it to a Limulus Amebocyte Lysate (LAL) test for detection of gram negative bacterial endotoxins. The test kit is manufactured by Whittaker Bioproducts, Walkersville, MD. Placebo-cocaine was an equal volume vehicle control.

Assay Procedures

LH Assay: Serum LH was determined in duplicate by the ImmuChem™ hLH IRMA method, using kits (Cat.#: 07-264-102) purchased from INC Biomedicals, Inc. (Costa Mesa, CA). The assay sensitivity was 0.2 mIU/ml and the intra- and interassay C.V.'s were 1.7% and 6.9%, respectively.

Prolactin Assay: Serum PRL was determined in duplicate by the ImmuChem™ hPRL IRMA method, using kits (Cat.#: 07-274-102) purchased

from INC Biomedicals, Inc. (Costa Mesa, CA). The assay sensitivity was 0.1 ng/ml and the intra- and interassay C.V.'s were 1.3% and 6.0%, respectively.

Testosterone Assay: Serum testosterone was determined in duplicate by the ImmuChem[TM] Testosterone IRMA method, using kits (Cat.#: 07-289-102) purchased from INC Biomedicals, Inc. (Costa Mesa, CA). The assay sensitivity was 2.8 ng/dl and the intra- and interassay C.V.'s were 6.2% and 7.7%, respectively.

Plasma Cocaine Analysis: Plasma cocaine levels were measured in duplicate using a solid phase extraction method described by SPEC Instruction Manual by Ansys with a Hewlett-Packard 5890 Series II gas chromatograph equipped with a capillary column and a Hewlett-Packard 5971 Series Mass Selective detector (Abusada et al., 1993). The assay sensitivity was 10 ng/ml and the intra- and interassay coefficients of variation were 2.0% and 2.9%, respectively.

Plasma Nicotine Analysis: Plasma nicotine levels were measured in duplicate using a gas chromatography-mass spectrometry method described by Jacob and co-workers (Jacob et al., 2000). The nicotine assay sensitivity was 1.0 ng/ml and the intra- and interassay CV's were 4.7% and 11.5%, respectively. Nicotine assays were conducted in the laboratory of Dr. Peton Jacob III at the University of California at San Francisco.

Data Analysis

Plasma cocaine and nicotine levels, and hormone values were analyzed using a two factor repeated measures ANOVA. If significant main effects were detected, one-way ANOVA's were performed to identify the time points that differed significantly from baseline within each group. Comparisons between the effects of active and placebo-cocaine, and between the effects of high and low nicotine cigarettes were also analyzed with ANOVA for repeated measures.

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Finally, the hormonal effects of acute cocaine administration and cigarette smoking were compared with ANOVA. The statistical significance of temporal covariance between LH, prolactin and testosterone and plasma cocaine levels or plasma nicotine levels were determined by regression analyses. The relationship between peak ratings of "sick" and peak plasma prolactin levels after cigarette smoking was also evaluated with regression analyses.

Estimates of the primary kinetic parameters (i.e., time to peak plasma concentrations (Tmax), peak plasma concentration (Cmax), and half-life ($t_{1/2}$) of plasma nicotine, plasma cocaine and LH were obtained directly from a nonlinear regression-estimation software program based on the Manual of Pharmacologic Calculations with Computer Programs using PHARM/PCS Version 4.2 (MicroComputer Specialist MCS, Philadelphia, PA). LH concentrations were fitted to a single dose, one-compartment model with bolus input, first order output, and elimination. Plasma concentrations were weighted by the reciprocal of the predicted concentrations. These pharmacokinetic parameters were analyzed with an ANOVA to determine whether there were any differences between the two drugs.

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RESULTS

Nicotine and Cocaine Plasma Levels [Figure 1]

Baseline Nicotine and CO Levels: Plasma nicotine levels before and after low and high dose nicotine cigarette smoking are shown in **Figure 1** (top panel). Before cigarette smoking, low levels of nicotine that averaged 1.22 ± 0.27 ng/ml and 1.33 ± 0.30 ng/ml were detected in the high and low nicotine cigarette groups, respectively. Baseline CO levels averaged 3.5 ± 1.4 ppm in the high nicotine cigarette group and 4.2 ± 1.1 ppm in the low nicotine cigarette group.

Nicotine Plasma Levels After Smoking: Nicotine plasma levels increased significantly within 2 min (or 4 puffs) ($P < 0.01$) and remained significantly above baseline throughout the 120 min sampling period. Peak plasma nicotine levels of 22.6 ± 3.4 ng/ml were detected within 14 min, and remained above 20 ng/ml between 12 and 20 min after cigarette smoking began. At the end of the 120 min sampling period, plasma nicotine levels averaged 8.22 ± 1.27 ng/ml. Plasma nicotine levels also increased significantly above pre-smoking baseline levels after smoking the low nicotine cigarette ($P < 0.01$). Peak plasma nicotine levels of 3.90 ± 0.77 ng/ml were detected at 12 min after low nicotine cigarette smoking began. At the end of the sampling period, plasma nicotine levels averaged 2.03 ± 0.49 ng/ml.

Cocaine Plasma Levels: Plasma cocaine levels after intravenous administration of 0.4 mg/kg of cocaine are shown in **Figure 1** (bottom panel). Cocaine plasma levels were maximal at 8 min after i.v. cocaine administration, and averaged 254 ± 18 ng/ml. Plasma cocaine levels gradually decreased over the remainder of the sampling period and averaged 58.8 ± 3.2 ng/ml at 120 min. The calculated half-life ($t_{1/2}$) of cocaine in plasma was 47.8 ± 0.5 min. No cocaine was detected in plasma after placebo-cocaine injection.

Figure 1 about here

Luteinizing Hormone Levels after Cigarette Smoking and I.V. Cocaine [Figure 2]

Baseline LH Levels in the Nicotine and Cocaine Groups: Before smoking, baseline LH levels averaged 5.14 ± 0.61 mIU/ml in the low nicotine cigarette group and 4.10 ± 0.44 mIU/ml in the high nicotine cigarette group. Baseline LH levels averaged 4.24 ± 0.64 mIU/ml in the placebo-cocaine group and 4.36 ± 0.87 mIU/ml in the active cocaine group. There were no significant differences in baseline LH levels before cocaine or placebo-cocaine administration or before low or high nicotine cigarette smoking.

Effects of Cigarette Smoking and Cocaine on LH: LH levels increased significantly within 14 min after high nicotine cigarette smoking in comparison to the pre-smoking baseline (P < 0.04) (Fig. 2, top panel). LH reached peak levels of 8.15 ± 0.89 mIU/ml when plasma nicotine levels averaged 20.3 ± 3.1 ng/ml. LH remained significantly higher than baseline for 40 min (between 20 and 60 min) (P < 0.01-0.004). The increase in LH was significantly correlated with the increase in plasma nicotine levels shown in Figure 1 (R = 0.642; P = .003). After low nicotine cigarette smoking, LH levels did not change significantly from baseline. LH levels were significantly higher after high nicotine cigarette smoking than after low nicotine cigarette smoking at 18, 20, 25 and 30 min post-smoking (P < .05) or (P < .03-.02).

After administration of 0.4 mg/kg i.v. cocaine, LH increased significantly within 20 min in comparison to baseline (P < 0.04) (Fig. 2, lower panel). LH reached peak levels of 5.8 ± 0.4 mIU/ml when plasma cocaine levels averaged 221.5 ± 16.9 ng/ml. LH remained significantly higher than baseline for 40 min (between 20 and 60 min) (P < 0.04). The increase in LH was significantly correlated with the increase in plasma cocaine levels shown in Figure 1 (R = 0.686; P = .0012). After placebo-cocaine administration, LH levels did not change significantly from baseline. LH levels were significantly higher after

cocaine than after placebo-cocaine at 18, 20, 25 and 30 min post-injection (P < .05).

Although high nicotine cigarette smoking and i.v. cocaine each produced significant increases in LH levels in men (**Figure 2**), cigarette smoking produced higher levels of LH than i.v. cocaine over the same time period. LH levels after high nicotine cigarette smoking were significantly higher than LH levels after i.v. cocaine at 20 though 60 min after drug administration (P < 0.02-0.04). Pharmacokinetic analyses showed that the Tmax and Cmax for LH was significantly higher after cigarette smoking than after i.v. cocaine (P <0.02-0.04). However, the $t_{1/2}$ of LH did not differ significantly after cigarette smoking and i.v. cocaine.

Figure 2 about here

Testosterone Levels after Cigarette Smoking and I.V. Cocaine [Figure 3]

Baseline Testosterone Levels in the Nicotine and Cocaine Groups: Baseline testosterone levels averaged 478.2 ± 38.45 ng/dl in the high nicotine cigarette group and 454.2 ± 37.3 in the low nicotine cigarette group, and these values were not significantly different. Baseline testosterone levels averaged 550.5 ± 30.1 ng/dl in the 0.4 mg/kg cocaine group and 541.17 ± 73.4 in the placebo-cocaine group, and these values were not significantly different. Also, there were no significant differences in baseline testosterone levels before active cocaine administration and high nicotine cigarette smoking.

Effects of Nicotine and Cocaine on Testosterone: After high nicotine cigarette smoking, testosterone levels tended to decrease, but these changes were not significantly different from baseline (**Figure 3**). Testosterone averaged between 454 ± 28.8 ng/ml and 434.7 ± 32.5 at 20 to 60 min after smoking when LH levels were significantly higher than baseline (see **Figure 2**). At the end of the 120 min sampling period, testosterone averaged 460 ± 34.5 ng/ml (Fig. 3, top panel).

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There were no significant differences in testosterone between the high nicotine cigarette and the low nicotine cigarette groups.

After cocaine and placebo-cocaine administration, testosterone levels were quite variable. There were no significant changes in testosterone in comparison to baseline after either cocaine or placebo-cocaine administration. Moreover, testosterone levels did not differ significantly at any time point in the cocaine and placebo-cocaine groups.

Figure 3 about here

Prolactin Levels after Cigarette Smoking and I.V. Cocaine [Figure 4]

Baseline Prolactin Levels in the Nicotine and Cocaine Groups: Prolactin levels before low nicotine cigarette smoking averaged 12.00 ± 1.14 ng/ml, and these were not significantly different from baseline prolactin levels before high nicotine cigarette smoking (13.88 ± 1.1 ng/ml). Prolactin levels before placebo-cocaine administration averaged 10.82 ± 1.23 ng/ml and these were not significantly different from baseline prolactin levels before cocaine administration (10.29 ± 1.13 ng/ml). There were also no significant differences in baseline prolactin levels before high nicotine cigarette smoking and active cocaine administration.

Effects of Nicotine and Cocaine on Prolactin: Nicotine and cocaine produced opposite effects on prolactin in men (**Figure 4**). After high nicotine cigarette smoking, prolactin increased rapidly to average 21.6 ± 5.21 ng/ml within 6 min after the smoking period ended. Prolactin remained significantly above baseline levels for 42 min ($P < 0.05$-0.03), then gradually decreased to baseline levels within 120 min after cigarette smoking began. The increase in prolactin levels was not significantly correlated with the increase in plasma nicotine levels shown in Figure 1 ($R = 0.433$; $P = .064$). Prolactin levels did not increase after low nicotine cigarette smoking and were significantly lower than after high nicotine cigarette smoking for 60 min (20 min – 80 min) ($P < 0.05$).

After i.v. cocaine injection, prolactin levels decreased significantly from baseline at 40 min and remained below baseline throughout the sampling period ($P < 0.05$-0.01). The prolactin nadir averaged 5.65 ± 0.45 ng/ml, a 31 percent decrease from the pre-cocaine baseline at 100 min post cocaine. At the end of the sampling period, prolactin levels after cocaine averaged 7.22 ± 1.67 ng/ml. The decrease in prolactin was significantly correlated with the increase in plasma cocaine levels shown in Figure 1 ($R = 0.652$; $P = .003$). In contrast, after placebo-cocaine administration, prolactin levels remained relatively stable across

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the sampling period, and ranged between 8.71 ± 1.6 ng/ml and 11.31 ± 1.48 ng/ml.. At the end of the sampling period after placebo-cocaine, prolactin levels averaged 9.87 ± 1.41 ng/ml. Prolactin levels after cocaine and placebo-cocaine were not significantly different at any time point.

Figure 4 about here

Subjective Effects of Cigarette Smoking and I.V. Cocaine [Figure 5]

"High" Reports after Cigarette Smoking: Subjects reported feeling "high" immediately after smoking began, but the "high" intensity diminished during the 12 min smoking period (Fig. 5, top left panel). Subjects reported feeling "high" after both low and high nicotine cigarettes, but the high nicotine cigarette produced significantly greater subjective effects than the low nicotine cigarette (P < 0.05). "High" reports were not significantly correlated with plasma nicotine levels.

"High" Reports after Cocaine: Subjects reported feeling "high" immediately after i.v. cocaine administration, and "high" reports continued to increase for 8 min, then gradually declined (Fig. 5, lower left panel). "High" reports were significantly correlated with plasma cocaine levels (P < .0003, P < .812). After placebo-cocaine, "high" reports did not differ significantly from the pre-cocaine baseline and were significantly lower than after active cocaine.

Comparison of "High" Reports after Cigarette Smoking and Cocaine: The "high" feeling induced by cocaine was significantly greater than after smoking a high nicotine cigarette (P < 0.05). The "high" ratings after cocaine were significantly greater than after cigarette smoking between 8 min and 40 min after drug administration began. Interestingly, the "high" ratings after smoking a low nicotine cigarette were significantly greater than "high" ratings measured after placebo cocaine administration.

"Craving" Reports after Cigarette Smoking: Baseline "craving" scores were elevated in both the low nicotine and high nicotine cigarette group, but these were not significantly different. When smoking began, reports of craving decreased immediately in both groups, and continued to decrease during the smoking period. "Craving" scores remained low for 8 min after the smoking period ended, then began to increase in both groups. The low nicotine cigarette group reported greater "craving" than the high nicotine cigarette group until 48 min after the end of the smoking period.

"Craving" Reports after Cocaine: In contrast to the cigarette smokers, cocaine abusers reported no "craving" for cocaine during baseline. After placebo-cocaine, subjects continued to report minimal "craving" for cocaine throughout the sampling period. However, after active cocaine, subjects reported relatively low levels of cocaine craving for 16 min, but there was considerable variability between subjects.

 "Sick" Reports after Cigarette Smoking: Only one subject reported feeling "sick" on one occasion at 2 min after smoking a low nicotine cigarette. Three subjects occasionally reported feeling "sick" after smoking a high nicotine cigarette. The maximal "sick" effect was reported at 18 min after smoking began and averaged 15.8 ± 9.7 on a VAS scale of 0-100. There was no significant correlation between peak reports of "sick" and peak levels of plasma prolactin.

Figure 5 about here

DISCUSSION

This is the first clinical study to compare the endocrine effects of i.v. cocaine and high nicotine cigarette smoking using a rapid (2 min) sampling procedure. The several similarities between the positive subjective and physiological effects of i.v. nicotine and i.v. cocaine were first reported by Jones and co-workers (Jones et al., 1999) and this study confirms and extends those findings. We found that both i.v. cocaine and high nicotine cigarette smoking produced rapid increases in LH and reports of subjective "high", whereas placebo-cocaine and low nicotine cigarettes had no effect on any endocrine or subjective report measures. Moreover, high nicotine cigarette smoking stimulated significantly greater increases in LH release than i.v. cocaine. In contrast, cocaine and high nicotine cigarette smoking had opposite effects on prolactin and reports of "craving". The possible significance of these findings and some implications of the similarities and differences between cocaine abuse and cigarette smoking are discussed below.

Cocaine and Nicotine Plasma Levels

Intravenous cocaine and high nicotine cigarette smoking each produced rapid increases in drug plasma levels, although differences in the routes of administration produced differences in the time required to reach peak plasma levels and the half-life in plasma. Detection of peak cocaine levels within 8 min after i.v. administration is consistent with previous studies in men (Mendelson et al., 2001). Peak nicotine levels were detected 14 min after cigarette smoking began. Differences in smoking procedures limit comparisons with previous

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studies, but the peak plasma nicotine levels achieved are comparable to those reported by others (Benowitz et al., 1990). The low nicotine cigarette also produced measurable levels of nicotine in plasma but these had no significant behavioral or endocrine effects.

Comparison of Cocaine and Nicotine Effects on LH

Cocaine and high nicotine cigarette smoking each stimulated rapid increases in LH that were significantly correlated with increases in plasma levels of cocaine or nicotine. The time course and duration of LH increases after cocaine are consistent with previous clinical reports (Mendelson et al., 2001; for review see Mello and Mendelson, 2002). This is the first report that cigarette smoking, like cocaine, stimulates LH in men. Moreover, cigarette smoking produced significantly greater and more sustained increases in LH than i.v. cocaine. These findings are not consistent with earlier clinical studies where no effect of cigarette smoking on LH was detected (Winternitz and Quillen, 1977; Seyler et al., 1986), and a number of procedural differences limit meaningful comparisons between those studies and the present study.   However, differences in the frequency and duration of sample collection after cigarette smoking could account for these discrepant findings. For example, no changes in LH were detected when a single sample was collected after each cigarette (Winternitz and Quillen, 1977) or at 5 min intervals for 25 min after smoking (Seyler et al., 1986). In the present study, samples were collected every 2 min for 20 min after smoking began, then at 5 or 10 min intervals for 100 min. Significant increases in LH were not detected until 18 min after smoking began. Differences in LH assay sensitivity also may have contributed to detection of

changes in LH in the present study; our LH assay sensitivity was 0.2 mIU/ml, but assay sensitivity was not reported in earlier clinical studies (Winternitz and Quillen, 1977; Seyler et al., 1986). In rats, both stimulation and inhibition of LH after acute nicotine or cocaine administration have been reported (for review see Fuxe et al., 1989; Mello and Mendelson, 2002).

Behavioral Correlates of Drug-Induced Increases in LH

The behavioral implications of rapid increases in LH release after i.v. cocaine and cigarette smoking are unclear. The significant correlations between increases in LH and plasma nicotine and plasma cocaine levels suggests that LH may contribute to the abuse-related effects of both drugs (for review see Mello and Mendelson, 2002). In young men, LH increases were temporally related to behavioral and physiological measures of sexual arousal (La Ferla et al., 1978). LH is essential for normal reproductive function (Yen et al., 1999), and high LH levels during the periovulatory phase in females may also be associated with increased sexual receptivity (Mello and Mendelson, 2002).

Cocaine induced a greater increase in subjective "high" ratings than high nicotine cigarette smoking. This finding differs from a previous study in which the "high" ratings after the highest dose of i.v. nicotine (3 mg/70 kg) were greater than after i.v. cocaine (40 mg/70kg or 0.571 mg/kg) (Jones et al., 1999). Moreover, the highest i.v. nicotine dose produced higher ratings on most of the subjective effects measures than i.v. cocaine (Jones et al., 1999). It is possible that i.v. nicotine has more potent subjective effects than smoked nicotine and is more comparable to i.v. cocaine. Drug plasma levels were not reported by Jones

and co-workers, so nicotine dose levels cannot be directly compared with the present study.

The pattern of "high" ratings also differed after i.v. cocaine and cigarette smoking. "High" ratings were significantly correlated with increases in plasma cocaine levels, but not with increases in plasma nicotine levels. Rather, the peak "high" rating occurred within 2 min after smoking began, then decreased during the 12 min smoking period. In contrast, the cocaine-induced "high" continued to increase for 4 min post-injection and remained above a VAS rating of 50 for 14 min.

LH and Testosterone Interactions after Cocaine and Nicotine

The significant increases in LH after cocaine and high nicotine cigarette smoking were not followed by changes in T levels. These findings are consistent with previous clinical reports where no changes in T were detected in men who smoked 6 cigarettes over 2 hr (Winternitz and Quillen, 1977) or who were given intranasal cocaine (Heesch et al., 1996). However, stimulation of LH is usually followed by stimulation of T, which in turn inhibits LH release (Yen et al., 1999). For example, in rhesus monkeys, when LH was stimulated by an opioid antagonist, nalmefene, T increased significantly within 40 to 50 min after significant increases in LH (Mello et al., 2000). It may be that the magnitude of LH increases in the present study were not sufficient to stimulate T. LH increased by 80 percent from baseline after i.v. cocaine and by 107 percent after smoking a high nicotine cigarette. Whereas in rhesus monkey, nalmefene stimulated LH increases of 147 and 187 percent from baseline were followed by significant increases in T (Mello et al., 2000). Differences in LH assay

procedures, as well as species differences limit these comparisons.

Alternatively, the bioactivity of the LH isoform stimulated by cocaine and by high

nicotine cigarette smoking might have been insufficient to alter T levels (cf.

Bergendah and Veldhuis, 2001). These data cannot directly address this

possibility, because LH levels were analyzed with radioimmunoassay.

High LH levels and low T levels were also measured in dogs after a period

of chronic smoking (Mittler et al., 1983). Based on analysis of liver 6 β-

hydroxylase activity, these findings were interpreted to suggest that chronic

cigarette smoking increased hepatic metabolism of testosterone (Mittler et al.,

1983). *In-vitro* studies indicate that both nicotine and cotinine inhibit LH-

stimulated steroidogenesis in isolated mouse Leydig cells (Patterson et al.,

1990).

Comparison of Cocaine and Nicotine Effects on Prolactin

Intravenous cocaine significantly decreased plasma prolactin levels within

50-120 min. These findings were anticipated because cocaine acts as an

indirect dopamine agonist, and prolactin release is under inhibitory dopaminergic

control (see for review Ben-Jonathan and Hnasko, 2001; Mello and Mendelson,

2002). In contrast, high nicotine cigarette smoking produced significant and

sustained elevations in prolactin. These findings were consistent with previous

clinical reports that cigarette smoking can result in increased prolactin levels

(Wilkins et al., 1982; Seyler et al., 1986; Kirschbaum et al., 1994). Intravenous

infusion of nicotine (0.5 µg/kg/min for 60 min) that yielded peak nicotine plasma

levels of 13.72 ± 1.7 ng/ml, also increased prolactin in some normal men

(Newhouse et al., 1990). Because prolactin is a stress labile hormone, malaise

or nausea induced by smoking could increase prolactin release (Seyler et al.,
1986). Although it is possible that discomfort associated with high nicotine
cigarette smoking may have increased prolactin levels, three of the six subjects
never reported feeling sick and peak prolactin levels were not significantly
correlated with peak reports of "sick". In rats, acute administration of nicotine by
intravenous, intracerebroventricular and intraperitoneal routes each consistently
resulted in dose-dependent increases in prolactin (Sharp and Beyer, 1986;
Hulihan-Giblin et al., 1990).

In the present study, prolactin increased to 50-78 percent above normal
baseline levels and average prolactin levels above 21 ng/ml were measured
between 18 and 60 min after high nicotine cigarette smoking. In men, the normal
range of prolactin is from 1-15 ng/ml and hyperprolactinemia is defined as a
persistent elevation of serum prolactin levels above 20 ng/ml (Yen et al., 1999).
The regulation of prolactin is very complex, and abnormalities in prolactin can
influence a variety of systems including immune function and reproductive
function. Chronic hyperprolactinemia can affect insulin and adrenal androgen
regulation and can result in decreased bone density (Yen et al., 1999).   The
extent to which the repeated induction of transient hyperprolactinemia in chronic
smokers may have adverse health consequences is unknown, but the incidence
and severity of osteoporosis is greater in cigarette smokers (CDC, 2001).

Possible Mechanisms of Cocaine and Nicotine's Hormonal Effects

The contrasting effects of cocaine and nicotine on prolactin presumably
reflect the fact that each drug increases extracellular dopamine levels by different
mechanisms as noted earlier in the Introduction (Kuhar et al., 1991; Watkins et

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al., 2000). Alternatively, high nicotine cigarette smoking may stimulate rapid release of prolactin by increasing endogenous opioids (see for review Pomerleau, 1998) which in turn may inhibit dopamine release (Crowley, 1988). Yet, endogenous opioid peptides also inhibit release of hypothalamic luteinizing-hormone-releasing-hormone (LHRH) which regulates LH release from the pituitary (Yen et al., 1999). A cigarette smoking-related increase in endogenous opioid peptides might predict a decrease, rather than an increase in LH.

A conceptual framework for reconciling concurrent stimulation of both LH and prolactin is provided by the pioneering studies of Yen and co-workers (Yen et al., 1985). In clinical studies, infusion of the opioid antagonist naloxone led to an increase in release of both LH and prolactin in synchronous pulses. It was hypothesized that opioidergic control of both LH and prolactin may be mediated by LHRH. Consistent with this hypothesis, administration of LHRH to men stimulated prolactin release (Yen et al., 1985). A close temporal concordance of LH and prolactin pulsatile release in men has been consistently observed (Veldhuis and Johnson, 1988). In the present study, high nicotine cigarette smoking induced significant temporally concordant increases in both LH and prolactin (R=0.911; P< .0001), and this could reflect nicotine's interactions with LHRH. Although the time course of LH increases after cocaine and cigarette smoking were similar, it is not clear if these drugs stimulate LH release by similar mechanisms. Deconvoluton analysis of cocaine's stimulation of LH suggested that the LH increase reflected a burst of LHRH (Mello and Mendelson, 2002). However, LHRH may be stimulated by norepinephrine, epinephrine and

neuropeptide y, either stimulated or inhibited by dopamine and gonadal steroid

hormones, and inhibited by endogenous opioid peptides (Yen et al., 1999). The

relative contribution of these complex and inter-related systems to the acute

effects of cocaine and nicotine on LH are unknown.

Acknowledgment

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Footnotes

Preliminary data were presented to the College on Problems of Drug Dependence in June, 2002.

Figure Legends

Figure 1:   **Plasma Nicotine and Plasma Cocaine Levels**. Plasma drug levels

(ng/ml) are shown on the left ordinates and time (min) is shown on

the abscissae. Points above BL were collected 10 min before drug

administration at time 0. Each data point is the average ($\pm$ S.E.M.)

of six subjects. The top panel shows plasma nicotine levels after

smoking a high nicotine yield cigarette (filled circles) and a placebo

cigarette (open circles). The 12 min cigarette smoking period is

indicated by the grey rectangle. The bottom panel shows plasma

cocaine levels after administration of 0.4 mg/kg, i.v. cocaine (closed

circles). The vertical line indicates when cocaine was administered.

Significant changes from the pre-smoking baseline are indicated by

asterisks (* = $P < 0.05$; ** = $P < 0.01$).   Daggers indicate points at

which plasma nicotine levels after smoking a low nicotine placebo

cigarette differed from plasma nicotine levels after smoking a high

nicotine cigarette ($\dagger$ = $P < 0.05$; $\dagger\dagger$ = $P < 0.01$).

Figure 2:   **Luteinizing Hormone Levels after Cigarette Smoking or i.v. Cocaine**

Administration. Plasma LH levels (mIU/ml) is shown on the

abscissae. Points above BL were collected 10 min before drug

administration at time 0. Each data point is the average ($\pm$ S.E.M.)

of six subjects. The top panel shows LH levels after smoking a

high nicotine yield cigarette (filled circles) and a placebo cigarette

(open circles). The 12 min cigarette-smoking period is indicated by the grey rectangle. The bottom panel shows LH levels after administration of 0.4 mg/kg, i.v. cocaine (closed circles) or placebo-cocaine. The vertical line indicates when cocaine or placebo-cocaine was administered. Asterisks indicate points that were significantly different from baseline (* = $P < 0.05$; ** = $P < 0.01$). Daggers indicate points at which LH was significantly different after high nicotine yield cigarette smoking than after placebo cigarette smoking, or when LH was significantly different after active cocaine than after placebo-cocaine († = $P < 0.05$; †† = $P < 0.01$).

Figure 3:   **Testosterone Levels after Cigarette Smoking or i.v. Cocaine Administration**. Plasma testosterone levels (ng/ml) are shown on the left ordinates and time (min) is shown on the abscissae. Points above BL were collected 10 min before drug administration at time 0. Each data point is the average (± S.E.M.) of six subjects. All other details are as described in the legend for Figure 2.

Figure 4:   **Prolactin Levels after Cigarette Smoking or i.v. Cocaine Administration**: Plasma prolactin levels (ng/ml) are shown on the left ordinates and time (min) is shown on the abscissae. Points above BL were collected 10 min before drug administration at time 0. Each data point is the average (± S.E.M.) of six subjects. All other details are as described in the legend for Figure 2.

Figure 5:   **Reports of "high' and "craving" after high and low dose nicotine cigarette smoking, and after cocaine and placebo-cocaine administration.**

Subjective ratings on a Visual Analogue Scale (0-100) are shown

on the left ordinates and time (min) is shown on the abscissae.

Points above BL were collected 10 min before drug administration

at time 0. Each data point is the average (± S.E.M.) of five or six

subjects. All other details are as described in the legend for Figure

2.

TABLE 1

SUBJECT CHARACTERISTICS

	Age ($\bar{x}$ ± S.E.)	Education ($\bar{x}$ ± S.E.)	BMI ($\bar{x}$ ± S.E.)	Years Cocaine Use ($\bar{x}$ ± S.E.)	Years Cigarette Smoking ($\bar{x}$ ± S.E.)	Cigarettes Smoked Per Day ($\bar{x}$ ± S.E.)
Cocaine (N=6)	24.7 ± 1.2	14.2 ± 0.7	25.8 ± 1.5	6.3 ± 1.3	NA	NA
Placebo Cocaine (N=6)	27.0 ± 1.3	15.0 ± 0.6	25.2 ± 1.5	7.2 ± 1.5	NA	NA
Nicotine Cigarettes (N=6)	23.5 ± 1.0	14.8 ± 0.7	22.9 ± 0.4	NA	5.0 ± 0.5	15.7 ± 1.3
Placebo Cigarettes (N=6)	23.8 ± 0.6	15.5 ± 0.3	24.4 ± 0.5	NA	5.0 ± 0.4	15.0 ± 0.8

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Figure 1.
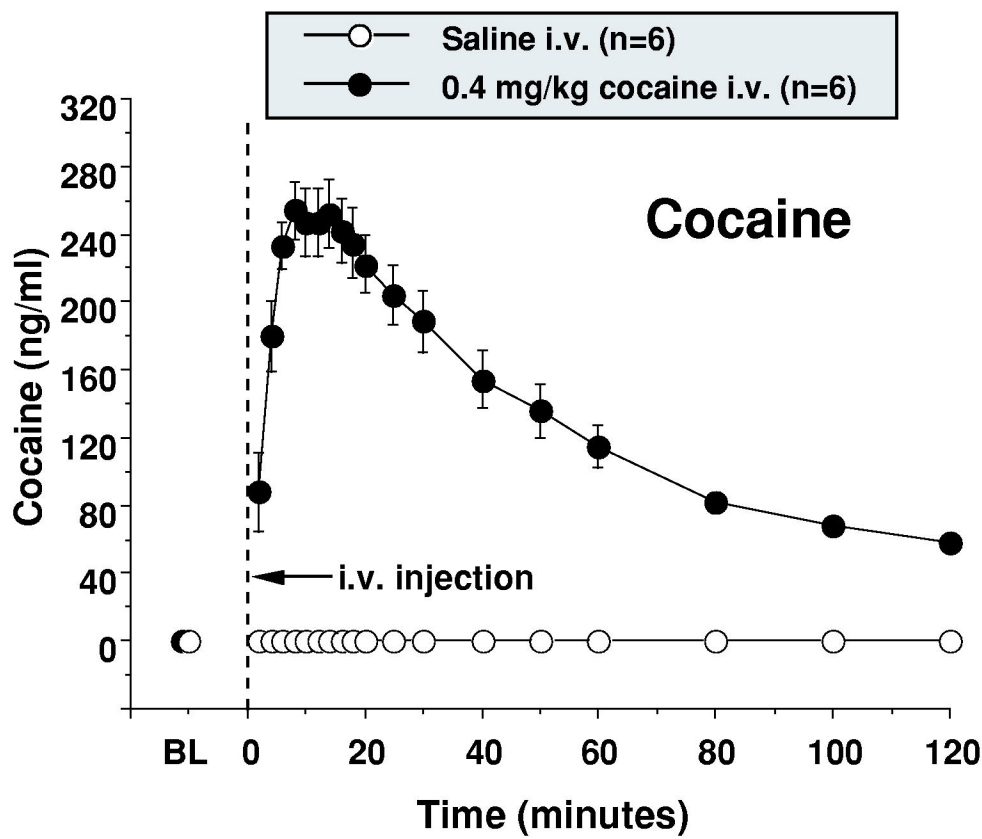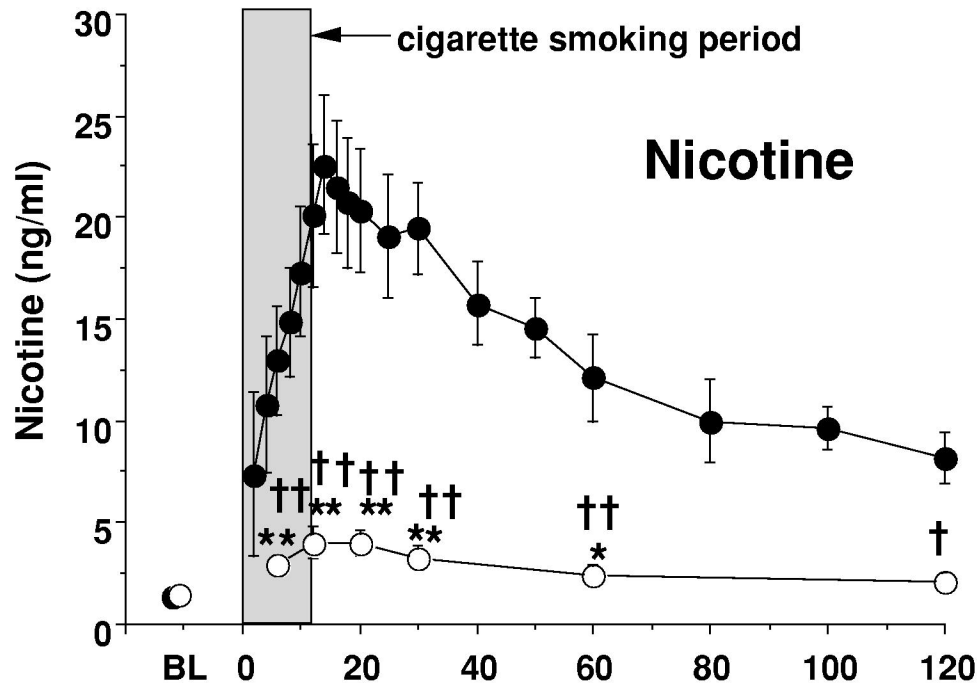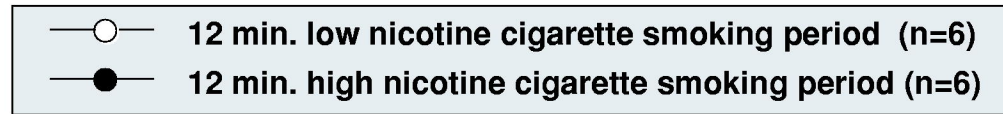
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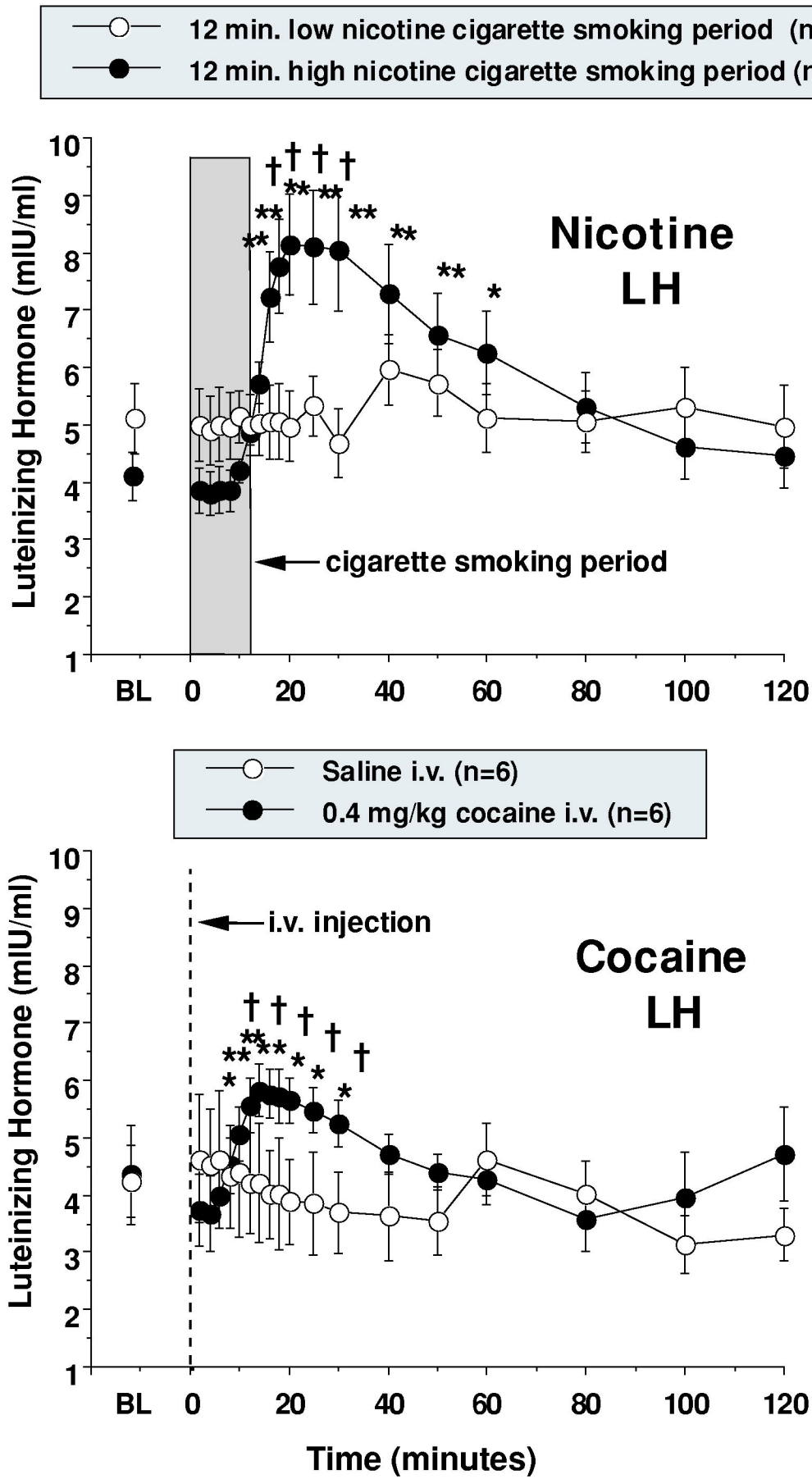
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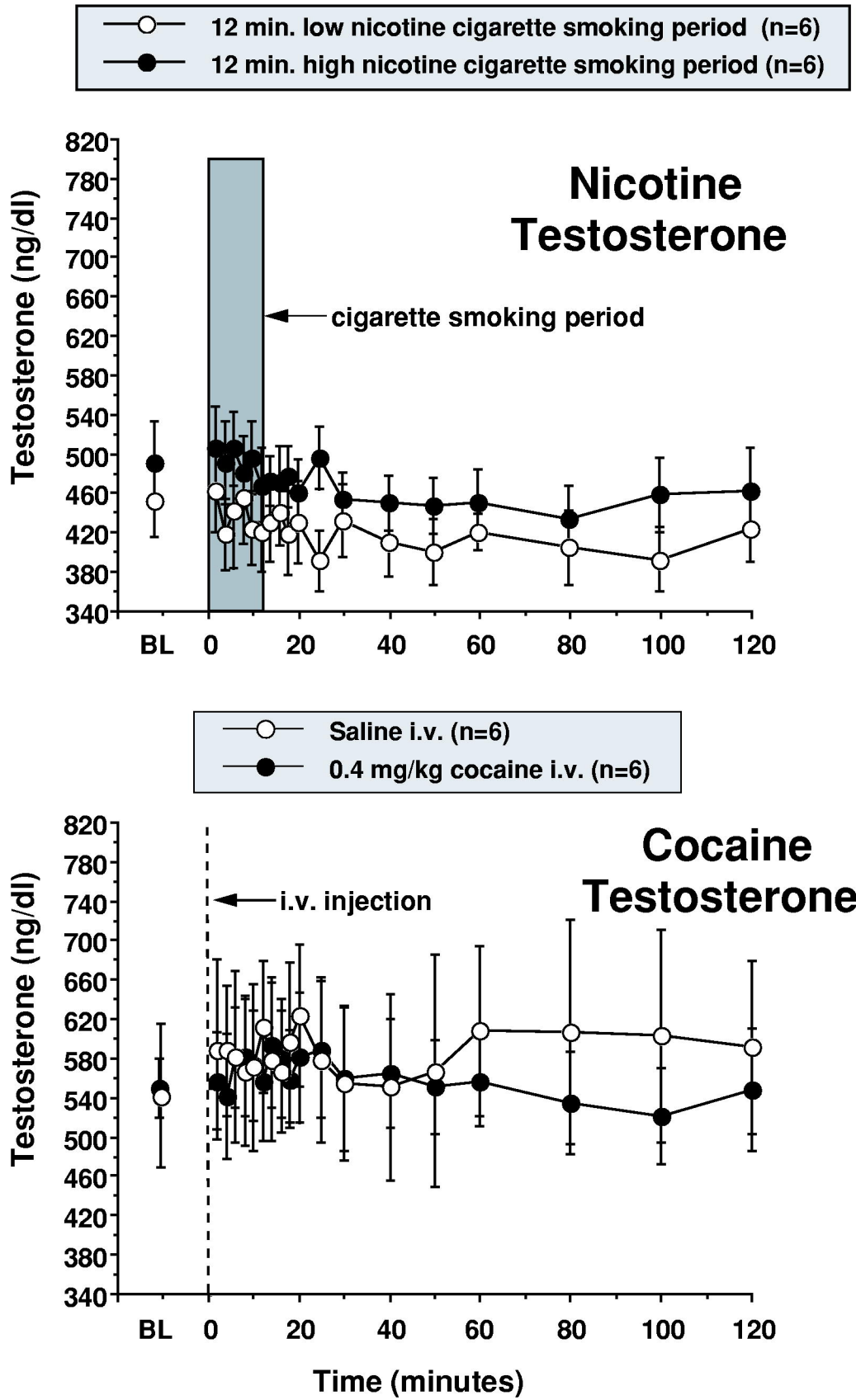
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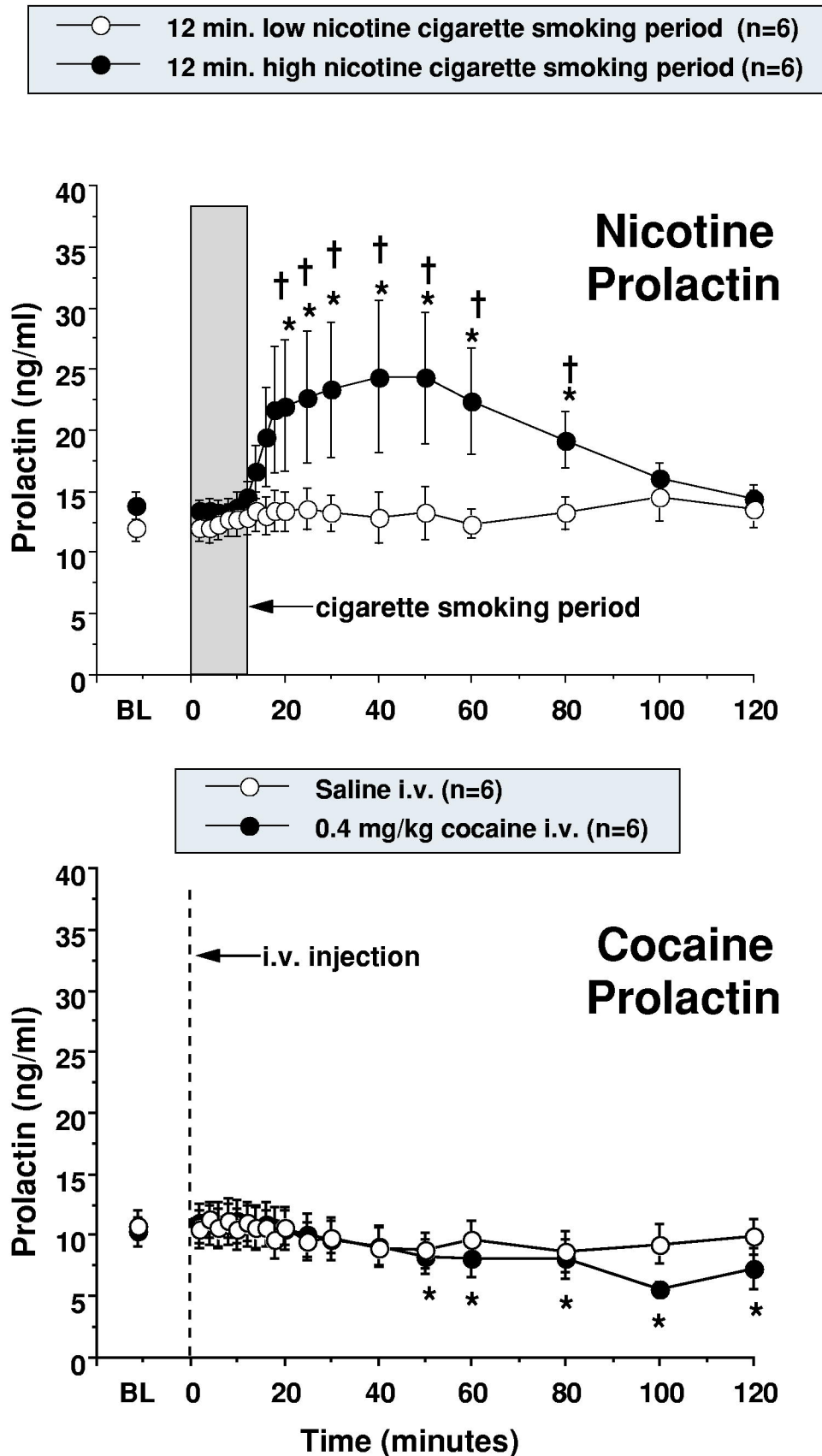
Figure 5.

How High Do You Feel?

- ○ 12 min. low nicotine cigarette smoking period (n=6)
- ● 12 min. high nicotine cigarette smoking period (n=6)

Nicotine

← cigarette smoking period

VAS (mm)

How Much Do You Crave Nicotine?

- ○ 12 min. low nicotine cigarette smoking period (n=6)
- ● 12 min. high nicotine cigarette smoking period (n=6)

← cigarette smoking period

VAS (mm)

How High Do You Feel?

- ○ Saline i.v. (n=5)
- ● 0.4 mg/kg cocaine i.v. (n=6)

← i.v. injection

Cocaine

VAS (mm)
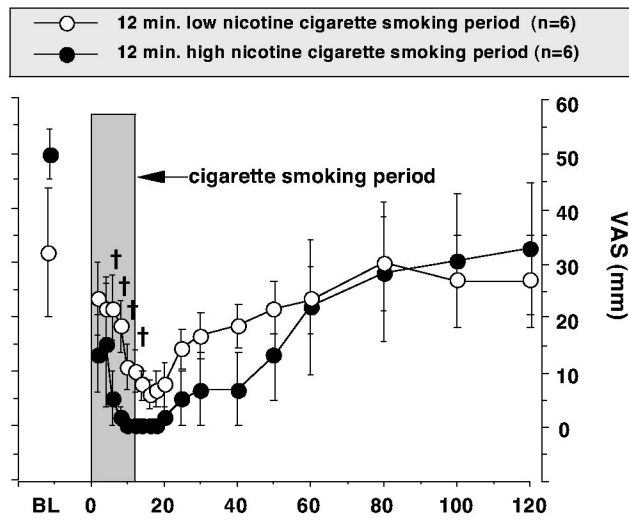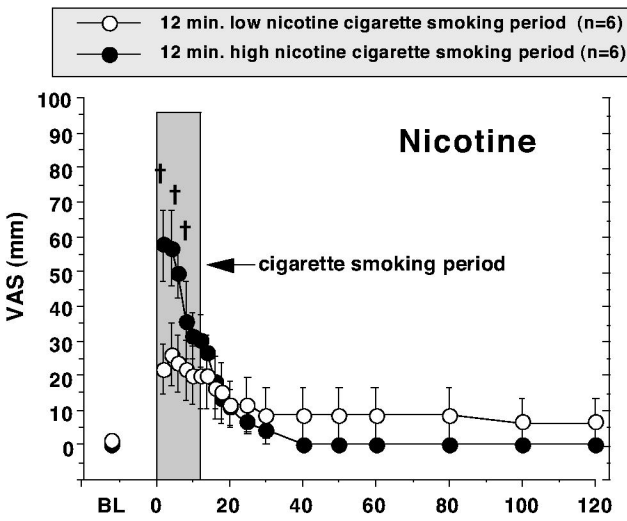
How Much Do You Crave Cocaine?

- ○ Saline i.v. (n=6)
- ● 0.4 mg/kg cocaine i.v. (n=5)

← i.v. injection

VAS (mm)

Time (minutes)