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Anorexic Activity of Cocaine and Coca Extract in Naive and Cocaine Tolerant Rats[1]

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VEE, G. L., G. B. FINK AND G. H. CONSTANTINE, JR. *Anorexic activity of cocaine and coca extract in naive and cocaine tolerant rats.* PHARMACOL BIOCHEM BEHAV **18**(4) 515–517, 1983.—Dose response curves for reducing limited access food consumption were determined for cocaine HCl IP, cocaine HCl PO, and whole *Erythroxylum coca* extract PO. The ED_{50}'s for cocaine HCl in drug naive rats were 19.6 mg/kg (IP) and 34.6 mg/kg (PO). When the dose of *E. coca* extract was expressed in terms of cocaine HCl content, the ED_{50} was 52.6 mg/kg (PO). When dose response curves were determined in rats that had received cocaine (45 mg/kg, PO) for 30 days, a shift to the right in the cocaine HCl curve (an ED_{50} of 98.4 mg/kg PO) indicated tolerance. However, the shift to the right was less for *E. coca* extract than for cocaine HCl. Although the anorexic activity of *E. coca* extract was less than that of an equivalent amount of cocaine in naive rats it was often more than that of equivalent doses of cocaine HCl in tolerant rats. Interaction with other constituents of *E. coca* extract appears to alter the potency of the cocaine content of the extract in different directions in naive and tolerant rats.

Cocaine HCl Coca extract Tolerance Anorexic activity

ERYTHROXYLUM coca leaves have been chewed by South American Indians for centuries to suppress hunger, thirst, fatigue, and for other purposes [9]. The *E. coca* leaves are chewed daily and used for long periods of time [9]. The pharmacological properties of *E. coca*, including the anorexic activity, have been attributed to the cocaine content [9,11].

Previously it was believed that cocaine was destroyed in the gastrointestinal tract and not very active if swallowed [11]. However, cocaine can be absorbed and has been found in the blood of *E. coca* chewers [8]. It has been reported in man that oral cocaine has the same subjective effects as intranasal cocaine [13]. In addition it has been demonstrated in animals that oral and intraperitoneal cocaine had similar activity on limited access food consumption [2, 3, 14].

The anorexic activity of cocaine is a well known property [12]. Tolerance to this activity has been demonstrated by intraperitoneal route of administration [7, 14, 15]. It has been proposed that cocaine is not the sole anorexigenic component of *E. coca* extracts since one cocaine-free fraction had significant activity in reducing food consumption [4]. No study to our knowledge has demonstrated a difference between an extract of the whole leaf and of cocaine alone on limited access food consumption.

In this study dose response curves were delineated for oral and intraperitoneal cocaine. In addition, the degree of tolerance developing from repeated oral administration of cocaine was determined. Finally the anorexic activity of cocaine and of whole leaf extract with equal cocaine content were compared in naive and cocaine tolerant rats.

METHOD

The subjects, male Sprague-Dawley rats, were individually housed in a temperature controlled environment with a 12 hr light-dark cycle. A liquid diet [6] was available twice daily for a one hour period. The subjects were allowed to acclimate to this feeding schedule until mean food intake varied by less than ten percent.

E. coca leaves were obtained from Peru with the generous assistance of Dr. Andrew Wiel of the Harvard Botanical Museum. A voucher specimen has been maintained. The dried leaves (1.07 kg) were ground in a Wiley mill and then percolated exhaustively with 95% ethanol until all alkaloids had been extracted (Dragendorf negative). Following evaporation of solvent at reduced pressure, the extract was suspended in water (500 ml) and lyophilized to assure that all ethanol was removed. The cocaine content was determined by a gas chromatography method [1] and verified on one occasion by gas chromatography-mass spectrometry. The cocaine content of the leaves was 0.565% and leaf extract had the equivalent of 39.3 mg cocaine HCl per gram of extract.

E. coca extract was suspended in normal saline with the aid of Tween 80 so that each rat would receive approx-

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imately 1 ml of preparation. Four dose levels were employed corresponding to a cocaine HCl content of 15, 30, 60, and 90 mg/kg. Cocaine HCl flakes USP were obtained from Mallinkrodt Chemical Co. Seven dosages (10, 15, 20, 30, 60, 90, and 120 mg/kg) of cocaine HCl were administered in 1 ml of 0.9% saline. All drug solutions, prepared immediately before each experiment, were given by gastric lavage or intraperitoneally.

In the anorexic testing procedure the acclimated rats were given 1 ml of saline vehicle at either 15 min (intraperitoneal) or 45 min (oral) before each feeding session for three days and thereafter except when the drug was given. The experimental design was a 5×5 Latin square with four drug dosages and one saline control dosage utilized [5]. A given dose of drug was administered on alternate afternoon sessions to eliminate possible drug carry-over effects. Each animal served as its own control to calculate changes in food intake. The data was statistically evaluated by ANOVA. Mean responses to different treatments were compared using Student's *t*-test. Log dose response regression lines were evaluated for linearity and compared for similarity using F tests. Similarity of slopes between two regression lines was determined by calculation of the 95% confidence limits for the *difference in slopes [10]*.

In the acute studies the rats had a mean weight of 300 g (±25 SEM). There were 4 to 5 rats in each of the five groups. Intraperitoneal dosages of cocaine HCl were given 15 minutes prior to feeding sessions, and four dosages (10, 15, 20, and 30 mg/kg) were employed. Oral dosages of cocaine HCl were given 45 minutes prior to feeding sessions in dosages of 15, 30, 60, and 90 mg/kg. Oral doses of *E. coca* extract were given in a similar manner and contained an equivalent amount of cocaine HCl.

In the chronic studies the rats (mean weight 240 g) were given an oral cocaine HCl dose of 45 mg/kg. This dose was administered 45 minutes before each feeding session, twice daily, for thirty days. The anorexic activity of oral cocaine and *E. coca* extract were again determined as described above except cocaine HCl (45 mg/kg) was given before feeding sessions instead of saline vehicle. There were 3 to 4 rats (mean weight 300 g) in each of the five groups. Cocaine HCl was evaluated at 30, 60, 90, and 120 mg/kg in order to complete dose response curves. The dosages of whole *E. coca* extract equivalent to 15, 30, 60, and 90 mg/kg cocaine HCl were tested for anorexic activity. To establish normal weight gain a control group of 10 rats received only the saline vehicle for thirty days.

RESULTS

As shown in Fig. 1, in the naive rats, orally administered cocaine HCl and *E. coca* extract, and intraperitoneally administered cocaine HCl exhibited linear log dose response curves (A, B, C) which did not deviate significantly from parallelism ($p<0.05$). The ED_{50}'s (dose that reduced food consumption by 50%) for cocaine IP, cocaine PO, and *E. coca* extract PO were 19.6 mg/kg (±0.4 SEM), 34.6 mg/kg (±0.6 SEM), and 52.6 mg/kg (±1.7 SEM), respectively, each ED_{50} differing significantly.

In the studies with cocaine tolerant rats, linear log dose response curves (D, E) were obtained with orally administered *E. coca* extract and cocaine HCl which were not parallel ($p<0.05$). However, log dose response curve of cocaine HCl in tolerant rats was parallel to that in naive rats ($p<0.05$) with ED_{50}'s of 98.4 mg/kg (±1.9 SEM) and 34.6 mg/kg, respectively. No rats died at the dosages studied throughout the experiments, but various stereotypic behaviors were exhibited.

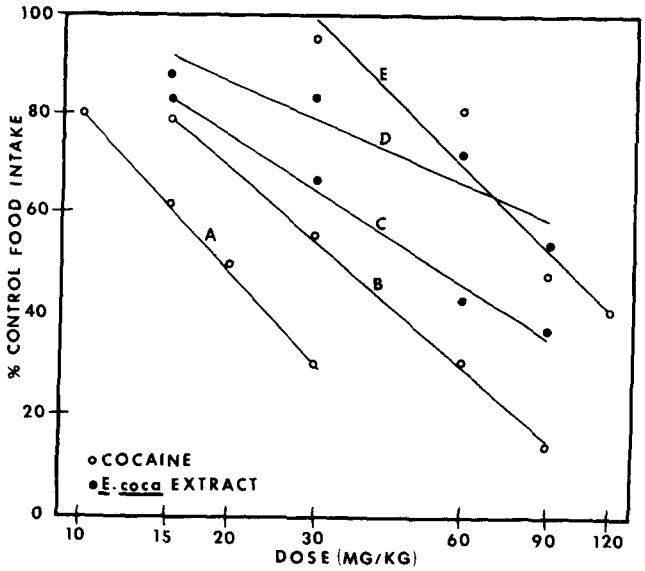

FIG. 1. Plots of mean responses (n=20–25) with corresponding regression lines in naive subjects treated with cocaine HCl IP (A), cocaine HCl PO (B), and *E. coca* extract PO (C); also in cocaine tolerant subjects given *E. coca* extract PO (D) and cocaine HCl PO (E).

Both the chronically treated group and the control group of rats gained weight over the thirty day period. During the first 15 days the cocaine treated group increased their weight by 10.0%, whereas the control group increased their weight by 17.2%. During the next 15 days the cocaine treated group increased their weight by 11.4%, whereas the control group increased their weight by 9.2%. After a period of 18 days there was no significant difference ($p>0.05$) in the food intake between control and treated subjects.

DISCUSSION

Increasing cocaine dosages gave a dose dependent reduction of food intake. The intraperitoneal dose that reduced food consumption by 50% (ED_{50}=19.6 mg/kg) compares well with the approximate ED_{50}'s, 14 mg/kg and 15 mg/kg, reported in previous studies [3,15].

Also, the present studies corroborate reports on the effectiveness of cocaine HCl by the oral route [3,13]. The oral ED_{50} was 76% greater ($p<0.05$) than the intraperitoneal ED_{50}. Differences from previous reports include the use of different strains of rats, number of subjects and observations, time of injection before feeding, length of feeding time, and possible toxic responses. The fact that the intraperitoneal experiments agreed with previous studies indicates that most differences from prior studies were probably minimal. The experimental design employed herein took into account the fact that alkaloid absorption from the gastrointestinal system may occur primarily via the small intestine [13], hence cocaine HCl was given orally 45 minutes before feeding rather than the 15 minute injection time for intraperitoneal dosages. Furthermore, the Latin square design allowed 20–25 observations to establish each point on the dose response curves.

To our knowledge this is the first report of the effect of whole *E. coca* extract on limited access food consumption. The dose response curve in naive subjects of *E. coca* extract, prepared to contain equivalent amounts of cocaine, was shifted to the right of that of oral cocaine HCl. The ED_{50} of *E. coca* extract was 50% greater ($p<0.05$). Thus the effect of *E. coca* extract in naive subjects could not be explained by the cocaine content alone and suggests that other constituents of *E. coca* extract affect the activity. Possibly other constituents affect the bioavailability of the cocaine in the extract or a less potent constituent decreases the cocaine activity.

This study also demonstrates the considerable tolerance that develops to repeated cocaine injections as previously noted [15]. The dose response curve to cocaine HCl after thirty days exposure demonstrated a considerable shift in the curve to the right in a parallel manner; the ED_{50} was 98.4 mg/kg, a 180% increase ($p<0.05$). However, greater activity was found when *E. coca* extract, which contained equivalent quantities of cocaine HCl, was given PO to the cocaine tolerant subjects, since the shift in the curve to the right appeared to be less than that observed with cocaine HCl and was not parallel to either the cocaine HCl curves or to the *E. coca* curve in naive rats. In contrast to the results in naive rats, *E. coca* extract, in low to moderate doses, exhibited greater activity than equivalent doses of cocaine HCl in cocaine tolerant rats. The substances responsible for the decreased effect of cocaine as a constituent of *E. coca* extract in naive subjects and for the enhanced effect of low to moderate doses of cocaine as a constituent of *E. coca* extract in cocaine tolerant subjects merit further research.

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