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The Effects of Cocaine Free Extracts of the Coca Leaf on Food Consumption and Locomotor Activity[1]

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BEDFORD, J. A., M. C. WILSON, H. N. ELSOHLY, C. ELLIOTT, G. COTTAM AND C. E. TURNER. *The effects of cocaine free extracts of the coca leaf on food consumption and locomotor activity*. PHARMAC. BIOCHEM. BEHAV. **14**(5) 725–728, 1981.—The effects of two cocaine free fractions of an ethanol extract of the coca leaf (*E. coca*) were compared using actometric and limited access food consumption paradigms in rats. Several intraperitoneal doses of two different fractions were tested in both procedures. Neither of the fractions produced any alteration in locomotor activity. Both fractions did, however, produce reductions in food consumption at two or more of the doses tested. The results clearly demonstrate that the coca leaf contains constituents other than cocaine that are biologically active.

Coca Cocaine Activity Food consumption rat

OVER the past one and one half years, our laboratory has undertaken the task of determining the behavioral effects of a number of extracts of the coca leaf (*E. coca*). In a recent paper [2], we indicated that the coca leaf may indeed contain one or more constituents in addition to cocaine which are behaviorally active in a number of situations. We demonstrated that a chloroform fraction of the crude ethanol extract of the coca leaf containing cocaine would reduce food consumption and increase locomotor activity. It was pointed out that this activity could have been accounted for by the cocaine contained in this extract. However, the water fraction of the crude ethanol extract of the coca plant which contained only trace amounts of cocaine would also suppress food consumption and this suppression could not be accounted for by the trace amounts of cocaine contained in this fraction. Finally, it was also shown that this water fraction did not alter locomotor activity.

The two fractions alluded to above were produced by partitioning a crude ethanol extract between chloroform and water with the chloroform fraction containing the bulk of the alkaloids including cocaine, while the water fraction contained the non-alkaloidal constituents.

In order to further separate the constituents of the water fraction, we partitioned this fraction between butanol and water. The purpose of the present paper was to determine the effects of these two fractions on food consumption and locomotor activity in rats.

METHOD

Subjects

The subjects were male Wistar rats (Harlan Industries, Cumberland, IN) weighing between 250–300 g at the start of the experiments. Water was freely available to the subjects in the feeding experiments and freely available to the subjects in the actometric experiments except when the subjects were in the actometers. All subjects were housed individually in galvanized steel suspension cages. Ambient temperature was maintained at 21±1°C and the light/dark cycle was 12 hr on, 12 hr off. Subjects in the actometric experiments had free access to food (Purina Rat Chow) up to 18 hours prior to being placed in the actometers. The deprivation conditions of the subjects in the feeding experiments are discussed in detail below.

Apparatus

Actometric testing was carried out in circular photocell actometers described previously [3]. The actometers were located in a dark, temperature controlled room. A white noise generator provided a continuous auditory environment. The feeding experiments were conducted in the subjects home cage utilizing specially designed feeders and ground rat chow (Purina®).

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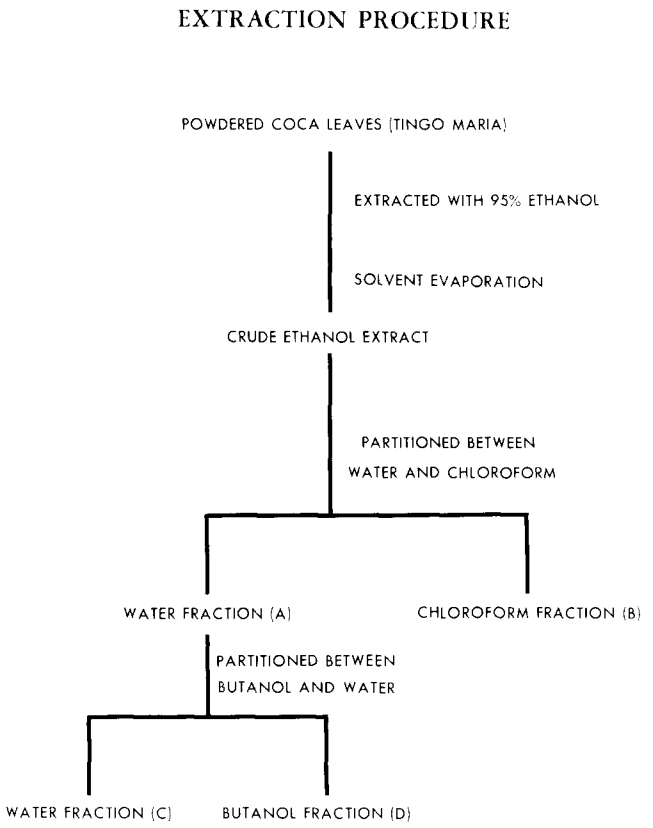

FIG. 1. Analytical procedure employed to separate the coca fractions studied. Tingo Maria refers to the area in Peru where the leaves were obtained.

Solution Preparation

Solutions were prepared on the morning of use. The analytical procedure used to obtain the coca extracts is outlined in Fig. 1 and described in detail below. Cocaine content of the solutions was determined by a method developed in this laboratory [5].

Coca leaves *(Erythroxylon coca)* obtained from the Tingo Maria area of Peru were powdered using a Wiley mill and exhaustively extracted by percolation with 95% ethanol. Evaporation of the solvent resulted in a crude ethanol extract containing all alkaloidal constituents of coca. The crude ethanol extract was then partitioned between water and chloroform resulting in two fractions (water fraction, A, and chloroform fraction, B). All water soluble compounds (e.g., water soluble alkaloids, quanternary ammonium compounds, sugars, cyclitols, glycosides, etc.) were contained in fraction A while fraction B contained the water insoluble constituents (e.g., alkaloids, terpenes, sterols, fatty acids, etc.). Fraction A was then washed repeatedly with chloroform to insure that all cocaine had been removed. After solvent evaporation, fraction A was then partitioned between butanol and water. The resulting two fractions, water (C) and butanol (D), were prepared for injection using sterile water. Injection (IP) volume was held constant at 2.0 ml/kg. Five doses (60, 120, 240, 480, 960 mg/kg) of fraction D and four doses (120, 240, 480, 960 mg/kg) of fraction C were tested in the feeding studies. Four doses of fraction D (60, 120, 240, 480 mg/kg) and one dose (480 mg/kg) of fraction C were tested in the actometric studies. Intraperitoneal doses were administered immediately prior to testing in both procedures.

PROCEDURES

Anorexic Testing Procedures

Following a 4 day acclimation period during which the subject had 24 hr access to chow (biscuit form) the subjects were randomly assigned to experimental groups (n=10). The subjects were then given 1 hr/day access to *ground* chow for 7 consecutive days. The amount of food consumed was measured after each days access period. On the next day all subjects within a test group were weighed and then dosed with the vehicle (distilled water) and immediately given 1 hr access to the ground chow. On the following day the same procedure was followed except that the appropriate test solution and dose were administered prior to assess to chow. Statistical comparisons between vehicle data and fraction data were accomplished via the Wilcoxom Matched-Pairs Signed-Ranks nonparametric test [4].

Actometric Testing Procedure

Following a four day laboratory acclimation period, the subjects were randomly assigned to individual groups (n=10). On the afternoon prior to testing the subjects food was removed (18 hr prior to test time) in order to control for deprivation state since this may influence locomotor activity. A session consisted of a 60 min determination of activity (recorded at 15 min intervals). The vehicle control for both fractions was distilled water. Statistical comparison between experimental and control groups was accomplished via the Mann-Whitney U test [4].

RESULTS

Neither fraction C nor D produced any systematic change in locomotor activity at any of the doses tested. Both fractions did produce a significant reduction in food consumption at two or more of the doses tested. Figure 2 presents the effects of the two fractions on food consumption. Fraction C significantly reduced consumption at both the 480 and 960 mg/kg doses. As can be seen fraction D significantly reduced food consumption at all doses tested and did so in a dose related manner. The 960 mg/kg dose of fraction D proved to be toxic as 6 of 9 animals treated with this fraction convulsed and died within approximately fifteen minutes of treatment.

DISCUSSION

Previous research from our laboratory had indicated that the coca leaf might contain constituents other than cocaine that were biologically active [1]. In these reports, we discussed the effects of a fraction (water layer) of the coca leaf containing only trace amounts of cocaine on food consumption. In the present paper this fraction, (following removal of all cocaine) was further partitioned between butanol and water. Neither of these fractions were observed to produce any change in locomotor activity. However, both fractions C and D did reduce food consumption. Fraction C did so only at the two highest doses tested (480 and 960 mg/kg) while

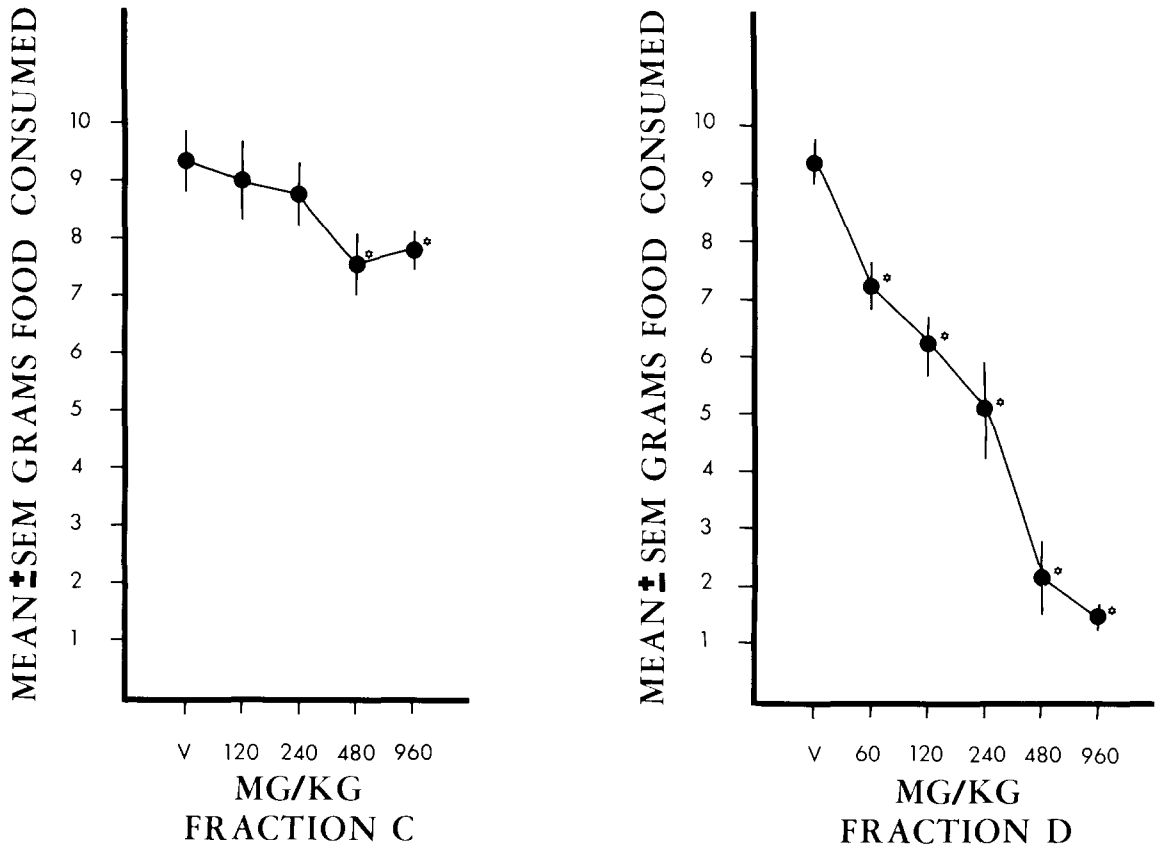

FIG. 2. Amount of food consumed as a function of the intraperitoneal dose of extracts C and D (see Fig. 1). *Indicates $p<0.05$.

fraction D did so at all doses tested. This data clearly supports our previous assumption that the coca leaf does contain constituents other than cocaine that are biologically active. Furthermore, this activity is, to a certain extent, qualitatively different from that observed with cocaine. Cocaine is a known appetite suppresant and in addition has been shown to produce marked increases in locomotor activity [1,2]. The two fractions reported here, like cocaine, reduced food consumption, however, unlike cocaine, neither produced a change in activity. In addition, as yet published data from our laboratories has demonstrated that these fractions do not share common stimulus properties with cocaine.

The similarity of effects observed with fractions C and D reported in the present paper are more than likely a result of incomplete fractionation. Thin layer chromatographic examination of both fractions used here showed that the constituents present are phenolic in nature. Through partitioning, most of the phenolic substances present in the original water fraction (see Fig. 1) were transferred into the butanol fraction. The water fraction, on the other hand contained trace amounts of the most polar phenolic constituents. These phenolic substances could be responsible for the effects observed with these two fractions.

One final point needs emphasis. The toxic effects observed with the highest dose of fraction D is quite striking, since this is the first report of any such effects from the coca leaf. This finding is even more important since this fraction is completely devoid of cocaine. The component responsible for this effect is unknown, however thin layer chromatographic analysis of this fraction has indicated there are a number of constituents that could be responsible for the effect.

In summary, the systematic reduction in food consumption observed with fractions C and D in the present paper clearly demonstrate that the coca leaf contains constituents other than cocaine that are biologically active. In addition, the toxic effects observed with fraction D coupled with its lack of stimulatory or depressant effects on locomotor activity, indicates the effects observed with the coca leaf cannot be wholly attributed to the cocaine content of the leaf.

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