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BRIEF COMMUNICATION

Chlordiazepoxide-Fluoxetine Interactions on Food Intake in Free-Feeding Rats[1]

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FELDMAN, R. S. AND W. C. SMITH. *Chlordiazepoxide-fluoxetine interactions on food intake in free-feeding rats.* PHARMAC. BIOCHEM. BEHAV. 8(6) 749–752, 1978. – Chlordiazepoxide, which blocks serotonin turnover, increased food intake; and fluoxetine, a serotonin agonist, decreased food intake. Administration of combinations of the two drugs showed an antagonistic dose-dependent relationship, implicating a satiety or hunger mechanims which is mediated by serotonin.

Chlordiazepoxide Fluoxetine Food intake Serotonin

PREVIOUS studies have investigated drugs in the benzodiazepine class with respect to their tendency to increase food intake and to increase efforts to obtain food. For example, chlordiazepoxide (CDP) increased food intake in food deprived rats when they were allowed to eat [14, 16, 18], in sated rats, and even in rats which have been stomach preloaded [16]. CDP also increased lever pressing for food (milk or food pellets) and in a conflict situation in which reward was accompanied by foot shock [2,14]. Oxazepam also increased eating of quinine adulterated food [14], and with pigs as subjects diazepam increased lever pressing for food pellets on a progressive ratio schedule. These and other studies were recently reviewed by Dantzer [4].

The mechanism of these benzodiazepine-induced increases in food intake and bar-pressing is not clearly understood. It seems unlikely that the drugs enhance general activity in a nonspecific way since CDP decreases spontaneous motor activity [11], and diazepam failed to increase bar-press responses when the animals had to press for water reinforcement [25]. An antianxiety explanation proposed by Margules and Stein [14] and Poschel, [17] states that the increases in food related behaviors may be attributable to the drug's effect of releasing behavior that is suppressed by fear. However, the antianxiety hypothesis does not explain results showing that food intake increased in situations where anxiety was probably at a minimum; i.e., when animals were tested in familiar environments (home cages) with the same food for control and test conditions [16, 18, 25]. These data lend support to an alternative hypothesis suggesting that the benzodiazepines specifically exert their effects on a food-specific mechanism [14,25]. We propose that these drugs either inhibit a satiety mechanism or activate or facilitate a hunger mechanism.

The benzodiazepines have been shown to influence systems mediated by norepinephrine, dopamine, GABA, and glycine [3], but it is likely that their behavioral effects are more directly mediated by serotonin (5-HT). Physiologically, CDP blocks 5-HT turnover and this may be indicative of reduced utilization of 5-HT [23,24]. Further, many behavioral effects of CDP are mimicked by 5-HT antagonists, and the effects of CDP are antagonized by 5-HT agonists [8, 10, 22]. Several studies have indicated a connection between 5-HT and food intake. For example, 5-HT depletion by intracranial injections of p-chlorophenylalamine (PCPA), which inhibits 5-HT synthesis, induced hyperphagia in rats [1]. 5-HT infused through hepatic-portal cannulae in rabbits decreased food intake in a free-feeding condition [19]. Peripheral administration of 5-HTP, the precursor of 5-HT, also decreased food intake, presumably by forming 5-HT in the brain by the influence of wisespread availability of the enzyme L-amino acid decarboxylase [9]. Furthermore, fenfluramine seems to exert a potent anorexigenic effect by releasing 5-HT and increasing its turnover rate [5,13].

A recently developed drug, fluoxetine (FXT) is a specific inhibitor of 5-HT uptake and therefore has the potential to enhance 5-HT mediated activity by prolonging the effect of 5-HT at the synapse [6, 7, 26]. FXT has been shown to

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decrease food intake in food deprived rats [9]. Our observations indicate that rats injected with FXT are somewhat sedated but they are easily aroused and show all typical rat behaviors, i.e., grooming, locomotion, and searching behavior. Therefore, the anorectic effect is not likely to be due to immobility; rather it appears to act primarily on the rats' food taking mechanism.

The present study investigated the effect on food intake when one of the benzodiazepines, CDP, was given alone and in combination with FXT to free-feeding rats. It was hypothesized that CDP would increase food intake, FXT would suppress it, and the two drugs in combination would have antagonistic effects.

METHOD

Animals

The animals were 10 male Sprague Dawley albino rats obtained from the Charles River Breeding Laboratories, and weighed between 400–500 g at the start of the experiment. Each rat was housed in a cage measuring 24 x 18 x 19 cm in a temperature-controlled room with an alternating 12 hr light–dark cycle. Purina rat pellets were available ad lib except during testing conditions when the weight of the food was controlled. Water was available at all times.

Procedure

The measurement of food intake was as follows. At the start of the daytime cycle (8:00 a.m.), the food was removed from the rat's cage. Three food pellets of known weight (between 14–17 g), were then placed in the cage. Four hr later (12:00 noon), the remaining food, plus the spillage, was weighed to the nearest 0.1 g.

During the first two weeks, the animals adapted to the light–dark schedule (on at 8:00 a.m., off at 8:00 p.m.), and were given a 4 hr food intake test on each day. On the basis of feeding during this period the Ss were matched into three groups (N=3, 3, 4). During the third week the rats were given five consecutive daily IP saline injections just before the food intake test to see if the injections would affect food intake. Since they did not, saline was omitted from further no-drug control tests to reduce the possibility of trauma during the long series of no-drug and drug test.

For the drug tests all drugs were injected IP in saline solutions such that the injected volume was the same for all doses. During the fourth, sixth, and eighth weeks CDP tests were given. For these tests the three groups received control food intake tests for two consecutive days followed on the third day by a drug test. During the drug tests each group received each of three dose levels of CDP (3.8, 7.5, and 15.0 mg/kg) at the onset of the test, one each week in a counterbalanced order. During the fifth, seventh, and ninth weeks, the rats were similarly injected with FXT. The doses of FXT were 2.5, 5.0 and 10.0 mg/kg. During the tenth through the nineteenth week, the rats were similarly injected at the beginning of the food intake test with combinations of CDP and FXT. Every week each group was injected with one of the possible nine CDP-FXT combinations until all groups had received all nine of the drug combinations in counterbalanced order.

RESULTS

Figure 1 shows the mean amounts of food eaten. A one factor ANOVA for repeated measures revealed highly

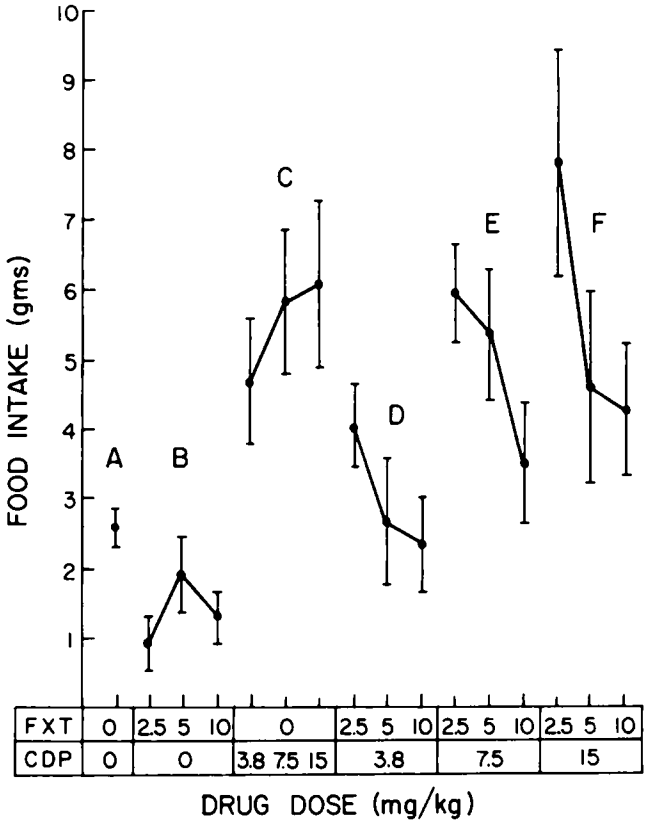

FIG. 1. Mean ± SEM food intake for chlordiazepoxide (CDP) and fluoxetine (FXT) treated rats. All measures for the 2-day no drug control tests which preceded drug tests were pooled and shown under A. B = FXT alone; C = CDP alone; D, E and F = FXT-CDP combinations.

significant main effects for CDP and FXT administered alone, $F(6,54) = 10.09$, $p<0.005$. In order to assess the effects of each dosage alone, sets of three multiple contrasts for each drug were carried out using the Bonferoni t procedure to control experimentwise error rate [15]. All tests were on 9 df. FXT significantly decreased food intake at the 2.5 mg/kg dose, $t=3.16$, $p<0.05$, and at the 10 mg/kg dose, $t=6.06$, $p<0.005$, but not at the 5 mg/kg dose. Thus, consistent with Goudie *et al.* [9], FXT seems to act as an anorectic drug.

Also, consistent with earlier studies, CDP significantly increased food intake over control tests. The differences were highly significant at 7.5 mg/kg, $t=3.33$, $p<0.025$, and at 15 mg/kg, $t=3.43$, $p<0.025$. At 3.8 mg/kg the difference was nearly significant, $t=2.41$; t-crit = 2.87, Bonferoni one-tailed t.

Figure 1 further shows the effect on food intake when both CDP and FXT are administered in combination. An analysis of these data by a two factor ANOVA for repeated measures showed that there was a significant main effect, CDP increased food intake when combined with FXT, $F(2,18)=6.57$, $p<0.01$, and FXT decreased food intake when administered with CDP, $F(2,18)=9.98$, $p<0.005$.

There was no significant interaction between CDP and FXT. This suggests that, for the most part, each drug

maintains its effect in the presence of the other and that there is a competitive interaction between them. However, Fig. 1 actually shows a non-monotonic relationship between FXT and food intake, while combining FXT with CDP yielded a dose response that was monotonic and decreasing. This discrepancy is probably due to a floor effect during the FXT alone tests when the animals were satiated and tested during the usual sleeping period. These factors no doubt made any dose related anorectic effect difficult to detect. But, the dose relationships became evident when increased food intake was induced by CDP.

One anticipated complication of this study was that CDP has a transient sedative effect that could have interfered with food intake during the drug tests. However, Fig. 1 shows that when CDP was given alone the higher doses which should have caused more sedation still increased food intake. Since repeated doses diminish the sedative effect [14,22] it would be expected that if sedation were a factor the order of administration of a given dose would be inversely correlated with the magnitude of food intake scores; or, the later a dose was given the less sedation it would cause, and more food could be eaten. On the other hand, even if this occurred the sedative effect for any given dose dissipates rather quickly and it is unlikely that sedation would have a significant effect on feeding during a 4 hr eating test. To examine the possibilities that sedation influenced eating behavior, all animals for each CDP dose were divided into low (0–5.1 g), medium (5.2–10.1 g), and high (10.2–15.1 g) food intake groups. For each dose the correlation between order of administration of the dose and level of food intake was tested by means of χ^2 tests. In a 3 x 3 table, df = 4, the critical value, $p<0.05 = 9.48$. The χ^2 values for 3.8, 7.5, and 15 mg/kg were 3.34, 2.90, and 3.53 respectively. Thus the data show no consistent relationhip between the two variables, and a possible role of sedation is ruled out.

DISCUSSION

CDP increased food intake in free feeding rats. Since CDP increased food intake in test conditions where anxiety was presumed minimal (food was not novel, animals tested in their home cage) the antianxiety hypothesis is not supported as explaining the increase in food intake.

Since CDP has effects on physiological systems that are mediated by norepinephrine, dopamine, GABA, and glycine as well as serotonin [3] it has been difficult to clearly establish which of these are important for the increased feeding effect. Testing the effects of CDP alone and in combination with fluoxetine, a specific blocker of 5-HT uptake, revealed the CDP-induced feeding was blocked by FXT in a dose-related manner. This suggests that CDP and FXT are competitively antagonistic with respect to their effects on food intake. Moreover, since FXT probably enhances 5-HT mediated synaptic transmission and CDP probably diminishes effects of serotonergic systems, the changes in food intake are quite possibly the result of alterations in one or more 5-HT mediated systems. This conclusion is buttressed by the findings that the anorectic drug fenfluramine causes an increased 5-HT turnover; cyproheptadine, a sertonergic receptor blocker, blocks fenfluramine induced anorexia [12], and cyproheptadine increases food intake, body weight and subjective feelings of hunger in man [21]. Also, Saller and Stricker [20] found that destroying 5-HT terminals with 5,7-dihydroxytryptamine led to hyperphagia and increased growth in rats when catecholamine levels remained normal. However, these authors suggested that the hyperphagia might be secondary or complementary to changes in growth hormone activity in the pituitary gland. Whether any manipulation of 5-HT mechanism directly or indirectly influences hormones involved in food intake will depend on future studies.

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