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Lack of Synergistic Feeding Enhancement by Systemic Clonidine and 8-OH-DPAT

DONALD V. COSCINA*†‡[1] AND ELIZABETH C. H. DE ROOY‡

Section of Biopsychology, Clarke Institute of Psychiatry and Departments of †Psychiatry and ‡Psychology, University of Toronto, Toronto, Ontario, Canada M5T 1R8

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COSCINA, D. V. AND E. C. H. DE ROOY. *Lack of synergistic feeding enhancement by systemic clonidine and 8-OH-DPAT.* PHARMACOL BIOCHEM BEHAV 44(4) 777–781, 1993.—To expand on recent suggestions that brain α_2-adrenergic and serotonergic systems may interact in their controls over feeding, three experiments were conducted to determine if combining clonidine and 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT) injections subcutaneously might produce exponential feeding enhancements greater than responses elicited by either agent alone. Ad lib fed adult male rats were tested in 2 × 2 drug designs in all studies. In Experiment 1, 30 μg/kg clonidine reliably enhanced feeding over a 6-hr test period. However, 250 μg/kg 8-OH-DPAT showed no signs of enhancing this response, but instead seemed to impede it as determined by analyses of cumulative intake curves. In Experiment 2, either 10 μg/kg clonidine or 250 μg/kg 8-OH-DPAT reliably enhanced 6-h interval intakes, but their combination again failed to synergize other than to interact with time in suppressing intake. To determine if feeding synergy might occur if subthreshold doses of each agent were combined, Experiment 3 tested 1 μg/kg clonidine and 15 μg/kg 8-OH-DPAT in 4-h feeding tests. Neither agent alone elicited reliable feeding as planned, but their combination also failed to stimulate feeding. These findings, combined with other recent work, do not support the possibility that previously demonstrated interactions between brain α_2-adrenergic and serotonergic systems extend to circumstances wherein enhancements of the former combined with suppressions of the latter might underly robust overeating responses.

Feeding 8-OH-DPAT Clonidine Interaction Synergy α_2-adrenergic Serotonergic Subcutaneous

PREVIOUS research has demonstrated that systemic injection of either the α_2-adrenergic agonist, clonidine (28,32,33) or the serotonin $(5\text{-HT})_{1A}$ agonist, 8-hydroxy-2-(di-*n*-propylamino) tetralin (8-OH-DPAT) (10,11,38), elicit dose-dependent feeding in satiated rats. In both cases, additional research has shown that such feeding appears to result from stimulating the respective adrenergic (14,25,26,29,33,36) or serotonergic (1,15,31,38) receptor types within the CNS. Because it has been suggested that brain α_2-adrenergic and serotonergic systems interact antagonistically in their controls over feeding (3,22,23,24,37), it might be expected that combined systemic injections of clonidine and 8-OH-DPAT would act synergistically to enhance feeding (see 6 and 9 for further elaboration of this). The present experiments were conducted to test this possibility.

METHOD

Subjects

A total of 70 adult male Sprague-Dawley rats (Charles River; Montreal, Quebec) were used in three separate studies. All animals were housed individually in stainless-steel cages within a temperature- (22 ± 2°C) and humidity- (40–50% relative) controlled colony room illuminated 12 h daily beginning at 0800 h. Throughout all investigations, rats had ad lib access to fresh Purina Lab Chow pellets (4% fat by weight) on their cage floors as well as tap water in bottles with sipper tubes.

Procedure

In the first experiment, 35 rats were adapted to our colony conditions for 3 weeks. Then, each rat was assigned to one of four groups of equal body weight and received the following double SC injections in the skin overly each rear flank: (a) Saline + Saline (n = 7); (b) Clonidine + Saline (n = 8); (c) 8-OH-DPAT + Saline (n = 8); (d) Clonidine + 8-OH-DPAT (n = 8). Immediately after these injections each rat was returned to its home cage in which a preweighed amount of fresh food pellets had been placed. Food intake was measured hourly for the next 6 h. All testing began between 1100 and 1200 h. Drug dosages used in this experiment were 30 μg/kg Clonidine hydrochloride (Sigma Chemicals; St. Louis, MO) and 250 μg/kg 8-OH-DPAT hydrobromide (Research

[1] Requests for reprints should be addressed to Dr. Donald V. Coscina, Section of Biopsychology, Clarke Institute of Psychiatry, 250 College Street, Toronto, Ontario, Canada M5T 1R8.

Biochemicals; Wayland, MA), each dissolved in saline and made up in a volume of 1 ml/kg.

In the second experiment, the 35 rats used in the first study were allowed to rest for 3 weeks, then were regrouped so that no rat received the same drug combination as before. The feeding effects of the same four drug combinations were again tested over 6 h, but this time the Clonidine dosage was reduced to 10 μg/kg. A pilot study in separate animals revealed that this dosage produced reliable feeding under our laboratory conditions.

In the third experiment, 35 new rats of the same age, sex, and strain were obtained and again adapted to the colony for 3 weeks. Then they were divided into the same four drug combination groups as already described, but this time the dosages of both drugs were lowered to subthreshold feeding levels (Clonidine = 1 μg/kg; 8-OH-DPAT = 15 μg/kg). The choice of these doses was determined from a small pilot study (n.s. of 3 to 4 rats each) in which 0.3, 1.0, or 3.0 μg of Clonidine and 15, 30, 60, or 120 μg 8-OH-DPAT were investigated. The doses of drugs selected were the highest that did not elicit reliable feeding separately in 4 h feeding tests compared to saline injection. The procedures employed for this last experiment were identical to those used in the two previous experiments except that intake was recorded hourly for only 4 h. This shorter observation period was used because pilot observations revealed feeding of lesser duration and magnitude under these lowered drug conditions.

Statistical Analyses

For each experiment, food intake was analyzed in two ways. For individual hourly intakes, separate three-way Analyses of Variance (ANOVA) (Clonidine × 8-OH-DPAT × Time) were performed, with repeated measures corrections made for the Time factor. For cumulative hourly intakes, two-way ANOVAs (Clonidine × 8-OH-DPAT) were conducted at selected time points along the testing continuum. For ease of reading, only p levels (two-tailed) of statistical tests have been reported.

RESULTS

The time course of feeding responses for groups in Experiment 1 are shown in Fig. 1. While separate hourly intakes were not high, the three-way ANOVA on interval responses-nonetheless revealed some highly reliable statistical results. Specifically, the main factors of Clonidine and Time were significant (see values summarized in Fig. 1). Although 8-OH-DPAT alone did not elicit feeding as a main effect, it did interact with Time to produce significance. However, the hypothesized interaction between Clonidine and 8-OH-DPAT was not observed, nor did both factors interact with Time to produce significance. The 2-way ANOVAs on cumulative intakes (see insert to Fig. 1) helped clarify these results. At 2, 3, or 4 h after injections, neither Clonidine nor 8-OH-DPAT alone produced reliable feeding effects. However, for the 2nd and 3rd h, both factors interacted significantly ($ps < 0.001$ and 0.035, respectively). Contrary to prediction, though, examination of the figure inset reveals that these results reflected a lower level of intake than would have been predicted based on the capacity of either agent alone to produce feeding. By the 4th h of testing, this effect had disappeared. Finally, by the end of the 6th h test, Clonidine alone had produced a reliable ($p < .005$) main effect on cumulative intake, but neither 8-OH-DPAT nor the interaction of both drugs were statistically significant.

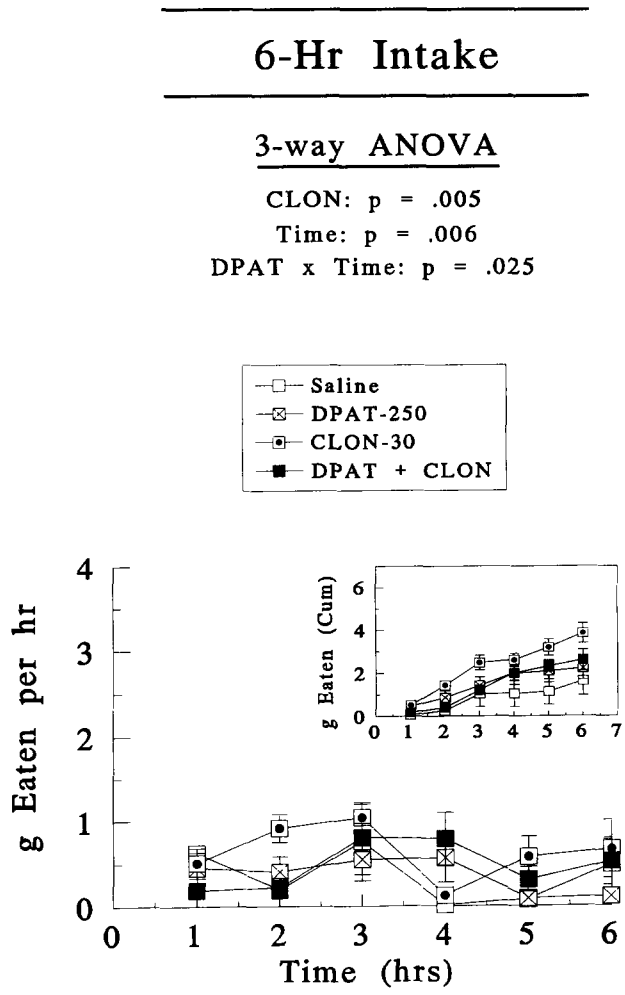

FIG. 1. Hourly (main graph) and cumulative (inset) intakes of groups tested in Experiment 1. Symbols defined in figure are as follows: Saline = double saline injections; DPAT-250 = saline + 250 μg/kg 8-OH-DPAT; CLON-30 = saline + 30 μg Clonidine; DPAT + CLON = both doses of the drugs. All injections were administered subcutaneously. The results of 3-way ANOVAs on hourly data are also summarized.

The time course of feeding responses for groups in Experiment 2 are shown in Fig. 2. In general, levels of intake were much higher in this study. The three-way ANOVA on separate hourly feeding responses revealed that all three main effects were statistically significant (see values summarized in Fig. 2). In addition, the Clondine × Time interaction approached significance. While no significant Clonidine × 8-OH-DPAT interaction was found, a reliable triple interaction was observed. However, examination of this graph revealed depressed interval intakes at h 3 and 5 compared to those seen for either drug alone. The selected two-way ANOVAs on cumulative intakes (see inset to Fig. 2) qualified these findings somewhat. While there were no reliable differences among groups for total intake 2 hrs after injections, either Clonidine or 8-OH-DPAT separately produced reliable cumulative feeding enhancements at 4 h ($ps < .032$ and .009, respectively) or 6 h ($ps < .013$ and .014, respectively) after injections. How-

ever, there were no reliable interactions between these drugs at any of these time points.

The time course of feeding responses for groups in Experiment 3 are shown in Fig. 3. For separate hourly intakes, the three-way ANOVA revealed only a significant Time effect (see values summarized in Fig. 3), with a trend toward a triple interaction. In keeping with these findings, separate two-way ANOVAs on cumulative intakes (see inset to Fig. 3) at 2, 3, or 4 h after injections revealed no main effects or interactions between them.

DISCUSSION

The results of these three experiments failed to confirm the possibility that combined systemic injections of Clonidine and 8-OH-DPAT might synergize with one another to produce powerful feeding responses. Aside from the literature cited in the Introduction, which indicated that such an expectation was not unreasonable for feeding, previous research in other fields also supports the possibility that such an interaction might occur because of interrelationships demonstrated to occur between α_2-adrenergic and serotonergic systems. As examples, the hyperglycemic and hypoinsulinemic effects of 8-OH-DPAT have been shown to be blocked by the α_2-adrenergic antagonistic, idazoxan, but not the α_1-adrenergic antagonistic, prazosin, nor the 5-HT_2 blocker, ketanserin (5). Furthermore, both clonidine and 8-OH-DPAT have been shown to depress the synthesis of endogenous 5-HT in frontal cortex and hypothalamus plus suppress cardiovascular functioning, albeit by slightly different mechanisms (35). In the mouse model of forced swimming, clonidine and 8-OH-DPAT have been shown to produce additive effects in blocking immobility (27), which is an index of clinical antidepressant activity. Finally, neurotoxic lesions of the median raphe nuclei that substantially depleted forebrain 5-HT have been reported to prevent

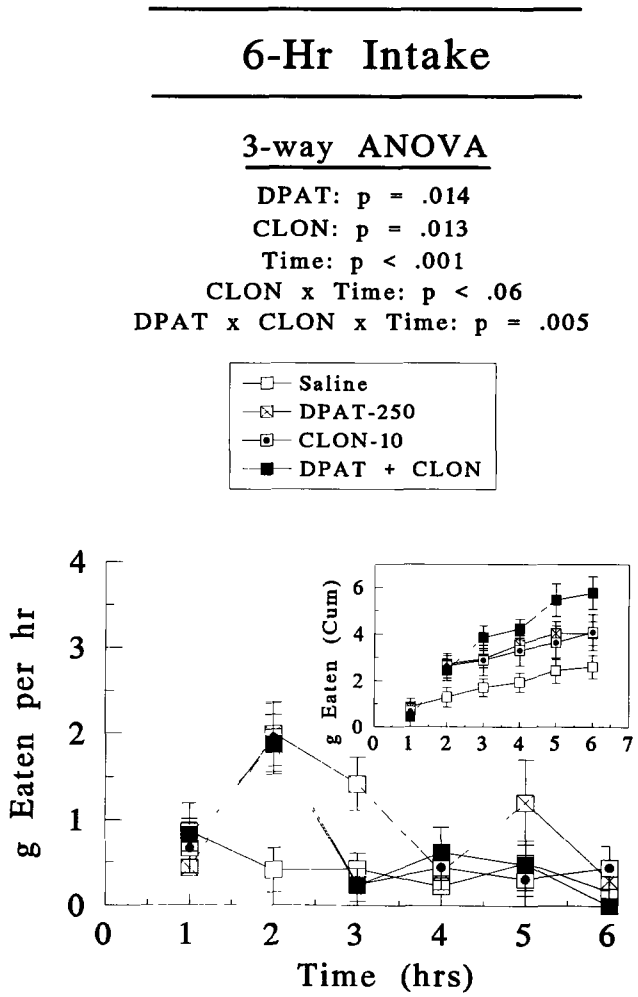

FIG. 2. Hourly (main graph) and cumulative (inset) intakes of groups tested in Experiment 2. Symbols defined in figure are as follows: Saline = double saline injections; DPAT-250 = saline + 250 μg/kg 8-OH-DPAT; CLON-10 = saline + 10 μg Clonidine; DPAT + CLON = both doses of the drugs. All injections were administered subcutaneously. The results of 3-way ANOVAs on hourly data are also summarized.

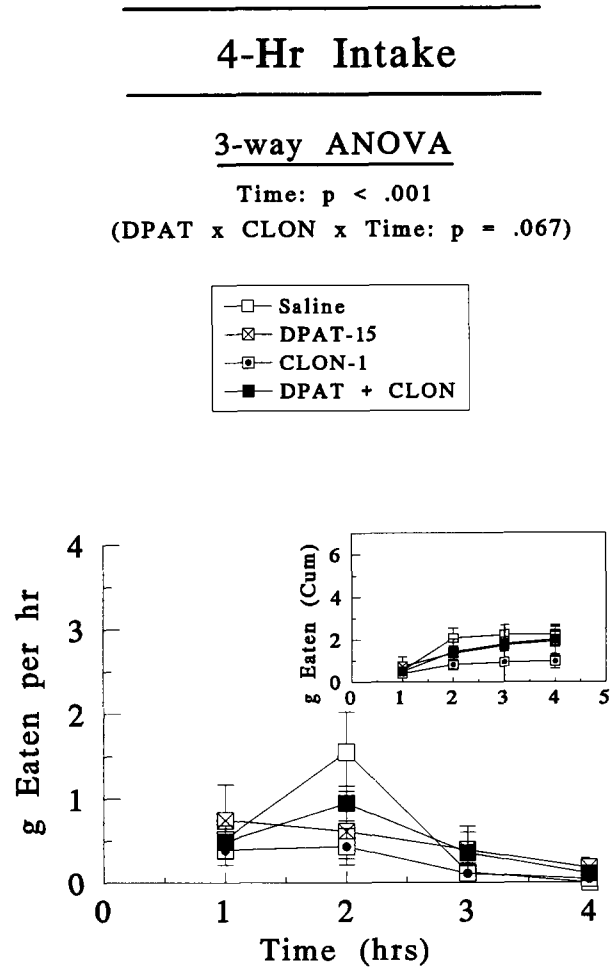

FIG. 3. Hourly (main graph) and cumulative (inset) intakes of groups tested in Experiment 3. Symbols defined in figure are as follows: Saline = double saline injections; DPAT-15 = saline + 15 μg/kg 8-OH-DPAT; CLON-1 = saline + 1 μg Clonidine; DPAT + CLON = both doses of the drugs. All injections were administered subcutaneously. The results of 3-way ANOVAs on hourly data are also summarized.

clonidine's suppressive effects on avoidance learning and locomotor activity (19).

Using moderate doses of both drugs, which have previously been shown to elicit feeding in their own rights (11,32), the results of Experiment 1 confirmed that 30 μg Clonidine could, indeed, produce feeding. In fact, the magnitude and time course of intakes we observed matched almost exactly those previously reported in a seminal paper on this topic (32). The failure of 250 μg 8-OH-DPAT to elicit unambiguous feeding enhancements was unexpected, since previous work has reported this dosage to reliably enhance intake (10,11), which indeed it did in the second experiment. Such inconsistent responding has been observed by us before (9), suggesting a certain fragility of this response. This may in part be related to the suggestion by others that the feeding elicited by 8-OH-DPAT is more related to its provocation of nonspecific behavioral arousal and/or gnawing responses (12,13,30), which might lead to more variable eating and/or be subject to meal fragmentation or disruption. More germane to the major question being addressed was the lack of any sign of synergistic interaction between both drugs. The only suggestion of an interaction was seen in 2 and 3 h cumulative intake responses. However, examination of the data (see inset to Fig. 1) reveals this interaction to be opposite to that predicted; that is, rather than enhancing Clonidine-induced feeding, 8-OH-DPAT suppressed it.

When animals were retested in Experiment 2 with a slightly lower dose of Clonidine (i.e., 10 μg) and the same dose of 8-OH-DPAT, much more reliable feeding was observed. Analyses of both interval and cumulative intakes confirmed the efficacy of each agent in producing feeding. However, both analyses failed to reveal a significant interaction between these main effects. Furthermore, the triple interaction found in the interval analysis again indicated a suppressant rather than enhancing effect after combining these drugs. Combined with the interaction findings of Experiment 1, this latter observation might be seen as supporting other data indicating that 8-OH-DPAT may possess some α_2-adrenergic antagonistic properties (4,8). However, since the latter findings were obtained from in vitro binding studies on peripheral tissues, their generalizability to the CNS properties of these agents and feeding behavior remains unclear.

Because the results of the first two studies provided no evidence of feeding synergy when liminal doses of both agents were used, the third experiment attempted to determine whether subthreshold doses might combine to elicit reliable feeding enhancements. As shown in Experiment 3, no evidence was obtained to support even that possibility.

In and of themselves, the results of these studies do not provide powerful evidence against the notion that brain α_2-adrenergic and 5-HT systems cannot interact in ways that might enhance food intake. For one thing, both drugs were administered systemically. Even though it has been proposed that both agents elicit feeding because of their central actions (see Introduction), using the systemic route nonetheless runs the risk of producing additional effects, which might separately or jointly have mitigated the possible occurance of enhancing intake (see 9 for discussion of some factors surrounding this). Another aspect of our design which might be thought of as contributing to negative findings was testing rats beginning 3 to 4 h into their light cycle. Previous work on the capacity of clonidine to elicit feeding after systemic (20) or direct injections into the paraventricular nucleus (PVN) of the hypothalamus (2) has shown that more robust feeding effects occur closer to dark onset. Similarly, the ability of 5-HT agonists to suppress feeding, particularly of carbohydrates, seems most likely to occur in the first large meal taken at dark onset (34). However, the fact that we were able to stimulate feeding of a standard chow diet, which is largely carbohydrate, with clonidine alone in both Experiments 1 and 2 and unambiquously with 8-OH-DPAT in Experiment 2 argues against this interpretation as substantially contributing to our results.

Perhaps more compelling support for our current findings derive from additional research which has attempted to demonstrate synergistic feeding enhancements by stimulating the α_2-adrenergic feeding response (26,36) elicited by injecting norepinephrine (NE) into the PVN of rats receiving 8-OH-DPAT either systemically (9) or directly into the dorsal or median raphe nuclei (7). In neither case were synergistic feeding enhancements observed. Combined with other recent data which demonstrate that PVN NE does not enhance feeding in rats depleted of hypothalamic 5-HT by prior injection of the neurotoxin 5,7-dihydroxytryptamine (6), the overall picture that emerges is one that does not favor the possibility that these two systems can, indeed, synergize to exaggerate food intake. Since such a mechanism is one which has been suggested to underlie the trait or state conditions contributing to excessive intake in certain human eating disorders (16,17, 18,21,24 [see 6 for additional discussion of this]), the negative findings reported here provide one more bit of evidence suggesting that some careful re-examination of this hypothesis may be required.

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