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European Journal of Pharmacology 419 (2001) 61–64

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Short communication

Anorectic effect of dehydroepiandrosterone combined with dexfenfluramine or thionisoxetine

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Received 29 March 2001; accepted 3 April 2001

Abstract

Free feeding rats given supplementary 1 h access per day to a palatable dessert test meal were tested for the anorectic effect of dehydroepiandrosterone alone or in combination with either the serotonin releasing agent dexfenfluramine or the norepinephrine uptake inhibitor thionisoxetine (LY 368975). Isobolographic analysis showed that the effect of dehydroepiandrosterone combined with either dexfenfluramine or thionisoxetine was within the range predicted for additivity of action. © 2001 Published by Elsevier Science B.V.

Keywords: Dessert test; 5-HT (5-hydroxytryptamine, serotonin); Norepinephrine; Isobologram; Additivity; Synergy

1. Introduction

Dexfenfluramine has been well studied both as a clinical weight loss agent and as an experimental tool for studying the role of serotonin mechanisms in the control of food intake (McTavish and Heel, 1992; Rowland and Carlton, 1986, 1988). The "off label" use of dexfenfluramine in combination with a catecholaminergic agent, phentermine (Wellman and Maher, 1999) led us to examine this and other combinations of anorectic agents in laboratory studies in rats. We reported the combination of low and statistically ineffective doses of dexfenfluramine and phentermine produced robust anorexia (Roth and Rowland, 1998). In later studies, we used isobolographic analysis (Berenbaum, 1989) to address the problem and found that the effect of this combination was statistically additive, but not synergistic (Roth and Rowland, 1999; Rowland et al., 2000). This distinction is important in development and testing of effective combination therapy. Thus, it was of relevance that a recent report claimed a synergistic anorectic effect of dexfenfluramine with dehydroepiandrosterone, an adrenal androgen, in obese Zucker rats (Gillen et al., 1999). Re-analysis of their data (see Section 4) raised concern about their conclusion. The present work uses the isobolographic method to evaluate the combined actions of dexfenfluramine with dehydroepiandrosterone in normal rats. We then extend this analysis to the anorectic action of a highly selective norepinephrine uptake inhibitor, [*R*]-thionisoxetine (LY 368975; Gehlert et al., 1995) given in combination with dehydroepiandrosterone.

2. Materials and methods

2.1. Animals and housing

These studies used male and female Sprague Dawley rats (Harlan Labs, Indianapolis), 5 months of age and weighing 400–480 g. All were housed singly in stainless steel cages ($17 \times 17 \times 21$ cm) suspended over absorbent paper. Food pellets (PMI 5001) and tap water were available ad libitum. The vivarium was maintained at 22 ± 2°C with lights on from 0600 to 1800 h. Intake studies were run during the middle of the day, 1300–1600 h, when spontaneous intake of standard food is very low.

2.2. General procedure

Rats were accustomed to receiving, in addition to chow ad libitum, a "dessert" of sweet milk. This was given every day at first then, after a few days when stable intakes were achieved, about four times per week. Milk was made fresh each day from 100 g sucrose and 100 g powdered

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0014-2999/01/$ - see front matter © 2001 Published by Elsevier Science B.V.
PII: S0014-2999(01)00958-X

nonfat milk (local supermarket brands) per liter tap water; caloric density is ~0.8 kcal/ml with macronutrient composition ~20% protein and 80% carbohydrate. The milk was presented at the front of the cage for 1 h in graduated 50-ml tubes fitted with stoppers and metal sipper tubes. Intakes were recorded daily until mean baseline intakes (recorded at 0.5 and 1 h) were stable (±10%). Rats were then divided into groups of six equated for baseline intakes. On the test days, rats were given the designated injections at the time stated before the daily dessert was made available. Intakes were recorded as before and were expressed as a percentage of each rat's baseline, which was computed as the average intake over 2–3 days preceding the test.

2.2.1. Dose–response function for dehydroepiandrosterone

Male rats ($N = 24$) were injected with either the vehicle (dimethylsulfoxide, DMSO 1 mg/kg i.p.) or with 50, 100 or 200 mg dehydroepiandrosterone/kg. Both chemicals were obtained from Sigma, St. Louis, MO. Exactly 1.5 h after the injection (in pilot work, delays of 0.5–2 h produced similar effects), the dessert was presented and intake recorded as described above after 0.5 and 1 h.

2.2.2. Dehydroepiandrosterone and dexfenfluramine

Fixed ratio (50:1) combinations of dehydroepiandrosterone and dexfenfluramine were used in 24 female rats. The combinations were 5:0.1, 15:0.3, and 25:0.5 mg/kg. Under identical test conditions (Roth and Rowland, 1999), the DI_{50} for dexfenfluramine was 2.5 (95% CI: 1.3–3.6) mg/kg. Dehydroepiandrosterone was given i.p. 1.5 h before the milk and dexfenfluramine (the HCl salt, a gift from L'Institut des Recherches Servier, in 0.15 M NaCl vehicle) was given s.c. 0.5 h before milk. Control rats received two vehicle injections.

2.2.3. Dehydroepiandrosterone and thionisoxetine

The procedure was as above, except that the norepinephrine uptake inhibitor, thionisoxetine (a gift from Dr. David Wong, Lilly Research Labs, Indianapolis, IN), was used instead of dexfenfluramine. Under identical test conditions (Rowland et al., 2000), the DI_{50} for thionisoxetine was 2.45 (95% CI: 1.5–3.4) mg/kg. In the main study, 24 female rats received treatments with dehydroepiandrosterone and thionisoxetine at dosages of 50 + 2, 25 + 1 and 12.5 + 0.5 mg/kg. Dehydroepiandrosterone was given i.p. 1.5 h and thionisoxetine was given s.c. 0.5 h before a 1-h intake test, with both agents dissolved in propylene glycol. A second, confirmatory study, using males and combination dosages of 25 + 1, 50 + 2 and 75 + 3 mg/kg, was performed using DMSO vehicle (as used in the prior studies) for both agents. In other pilot work, we ascertained that the effect of dehydroepiandrosterone was comparable using the DMSO and propylene glycol vehicles.

2.3. Data analysis

Data were analyzed by one-way ANOVA with Bonferroni post hoc comparisons ($P < 0.05$) using the SigmaStat software package. The DI_{50} and 95% CI values were calculated by linear regression and, to assess whether the effect of drug combinations differed from additivity, isobolograms were plotted.

3. Results

3.1. Dose–response for dehydroepiandrosterone

Dehydroepiandrosterone produced a dose-related inhibition of dessert intake. The mean (±S.E.) intakes in 0.5 h after pretreatment with either 0, 50, 100 and 200 mg dehydroepiandrosterone/kg were 91 ± 10%, 56 ± 8%, 30 ± 6% and 43 ± 10% of baseline (17.6 ± 1.3 ml), with all drug doses significantly ($P < 0.05$) lower than vehicle. Intake after 1 h was similar. Because the anorexia was maximal after 100 mg/kg, the percentage intakes from the 0, 50 and 100 mg/kg groups only were analyzed by linear regression. At both 0.5 and 1 h, the regressions were highly significant ($r > 0.99$) and the interpolated DI_{50} values were 65 (95% CI: 36–96) and 81 (95% CI: 58–100) mg/kg, respectively.

3.2. Combination of dexfenfluramine and dehydroepiandrosterone

The results of the combination of fixed ratio (50:1) doses of dehydroepiandrosterone and dexfenfluramine are shown in Fig. 1A. There was a dose-related suppression of intake, with the two higher doses significantly different from control and the linear regression highly significant ($r > 0.96$). The estimated DI_{50} values were similar at 0.5 and 1 h, and the average was 31.7 mg dehydroepiandrosterone/kg combined with 0.63 mg dexfenfluramine/kg.

The isobologram of these results is shown in Fig. 1B. The DI_{50} of the combination estimated above is percentage lower than that predicted by the line of additivity joining the DI_{50} values for each drug alone and, at this 50:1 ratio, would be 37.5 mg dehydroepiandrosterone/kg and 1.25 mg dexfenfluramine/kg. This is within the range of additivity as defined by the 95% CIs of DI_{50}s of the component drugs.

3.3. Combination of thionisoxetine and dehydroepiandrosterone

Results of the combination of fixed ratio (25:1) doses of dehydroepiandrosterone and thionisoxetine are shown in Fig. 1C. The two higher combinations significantly suppressed intake by >50% but the lowest combination was

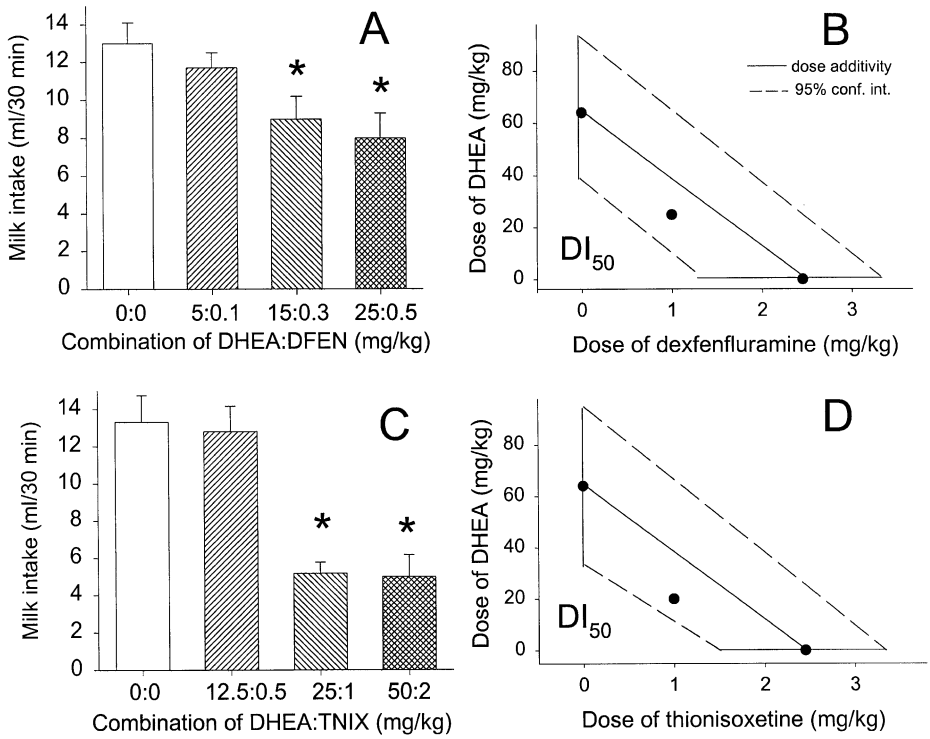

Fig. 1. Panel A: Mean (±S.E.) milk intakes of rats treated with vehicle or with three fixed ratio combinations of dehydroepiandrosterone (DHEA) and dexfenfluramine (DFEN). * $P < 0.01$ vs. vehicle. Panel B: DI_{50} values and 95% CIs for DHEA alone (*y*-axis) and DFEN alone (*x*-axis; data from Roth and Rowland, 1999) are joined by the theoretical line of additive effect. The dashed lines represent the 95% CI boundaries for additivity of effect. The DI_{50} of the combination, determined from the data in panel A, is also shown and falls within the trapezium of additivity. Panel C: As for panel A, but with fixed ratio combinations of DHEA and thionisoxetine (TNIX). Panel D: As for panel B, showing DI_{50} values and 95% CIs for DHEA alone (*y*-axis) and TNIX alone (*x*-axis; data from Rowland et al., 2000) joined by the theoretical line of additive effect. The 25:1 dose, which is actually greater than the DI_{50} of the combination (panel C), is also shown and falls within the trapezium of additivity.

ineffective, and the dose–effect curve is non-linear. However, the DI_{50} must lie between the two lowest combination doses, and slightly less than the DI_{50} predicted (32.5 mg dehydroepiandrosterone and 1.2 mg thionisoxetine/kg) from the isobologram (Fig. 1D), but within the 95% CI of additivity. The second study using males gave similar data (not shown) with the highest dose (75 + 3 mg/kg) being the most effective (intake 23 ± 4% of baseline).

4. Discussion

These results confirm that the adrenal steroid dehydroepiandrosterone produces a dose-related suppression of food intake. Previous studies have differed in several aspects, including the use of Zucker rats and/or a high fat meal (no deprivation) or after deprivation (Gillen et al., 1999; Pham et al., 2000). Although those studies did not formally report a DI_{50} for dehydroepiandrosterone, it can be estimated to lie between 50 and 100 mg/kg, consistent with the present result using a fat-free sweet dessert in outbred rats. In the final study, the effect of dehydroepiandrosterone was similar using either propylene glycol or DMSO as vehicle. The acute anorectic effect of dehydroepiandrosterone thus is robust and relatively insensitive to these procedural aspects.

Our conclusion concerning the additive effect of dexfenfluramine and dehydroepiandrosterone differs from the interpretation of synergy in a previous paper (Gillen et al., 1999). We re-examined their results (Table 1 of that report) and came to a different conclusion. The authors used only one dose of dehydroepiandrosterone (50 mg/kg) and the average (from four groups) suppression of intake was a modest 15%, in no case significantly different from controls. In our study, 50 mg dehydroepiandrosterone/kg produced a larger (35%) suppression, also not reliably different from vehicle, but consistent with the present DI_{50} of ~65 mg/kg. They used four doses of dexfenfluramine and found a non-linear dose–effect curve, with no suppression at 1 and 2 mg/kg and ~50% at 3 and 4 mg/kg. The DI_{50} is hard to ascertain from those data but appears comparable to the value (2.5 mg/kg) used in this study. The lowest combination doses that they used (50 + 1 and 50 + 2 mg dehydroepiandrosterone + dexfenfluramine/kg) produced suppressions of 68% and 48%, respectively, which according to the isobologram of the present study (Fig. 1B) would be slightly less than additive. Thus, we do not support the claim made by Gillen et al. (1999) concerning synergy of anorectic action of these agents.

The combination of thionisoxetine and dehydroepiandrosterone was additive also (Fig. 1D). Note that the point drawn in Fig. 1D is a conservative estimate of the true DI_{50} because of the non-linearity of the dose–effect curve. Pham et al. (2000) reported decreases in norepinephrine concentrations in lateral hypothalamus and paraventricular nucleus following administration of dehydroepiandrosterone to lean Zucker rats, suggesting that norepinephrine release had occurred. In this case, functional interaction with the uptake inhibitor, thionisoxetine, would be expected. The present behavioral data are in the direction, but statistically fall short, of behavioral synergy. Further, in immunocytochemical studies, we found that the number of neurons expressing the inducible transcription factor Fos was no greater in any brain region examined (including the paraventricular and lateral parabrachial nuclei and nucleus of the solitary tract) following dehydroepiandrosterone alone than in combination with either dexfenfluramine or thionisoxetine.

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