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Anorectic efficacy of the fenfluramine/phentermine combination in rats: additivity or synergy?

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Abstract

Fenfluramine + phentermine was a widely used combination for weight loss. Fenfluramine and phentermine are believed to act via serotonin and catecholamines, respectively. To what extent these drugs interact has not been well-established. We compared the anorectic efficacy of a range of doses of the combination (using dexfenfluramine instead of fenfluramine) relative to a range of doses of the individual drugs in 90 min sweetened milk intake tests in deprived and nondeprived rats. Results were plotted on isobolograms to determine whether the anorectic effects of the combination were either additive or synergistic. Collectively, the isobolographic analysis revealed that: (1) under acute conditions, dexfenfluramine and phentermine interact for the most part in a synergistic manner, and (2) with the exception of phentermine alone, deprivation state at time of testing did not alter the efficacy of the anorectics. These findings suggest that combined drug treatment for obesity is a theoretical approach that merits further investigation. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Dexfenfluramine; Phentermine; Synergy; Deprivation; Anorectic agent

1. Introduction

While there are many studies on the effects of individual anorectic agents on intake and body weight in humans and nonhumans, very little is known about the efficacy and safety of drug combinations. This is troubling considering the frequency with which combinations of diet drugs have recently been either prescribed or self-administered and their potential for adverse side effects. For example, with respect to 'fen/phen', the combination of fenfluramine (a serotonergic) and phentermine (a catecholaminergic), it has been estimated that in 1996, the total number of prescriptions in the United States exceeded 18 million. However, the use of this combination was sharply curtailed when it was found that fenfluramine and its active enantiomer dexfenfluramine may be exerting untoward effects on the cardiovascular system and that these effects may be exacerbated by the addition of phentermine (Connolly et al., 1997).

The withdrawal of the fenfluramines from the market has left many overweight individuals and their clinicians without effective pharmacological tools. In spite of the potential health risks associated with fenfluramine/phentermine, the combination *was* highly effective at promoting weight loss. In a long-term study of weight control, patients taking fenfluramine/phentermine had lost 15–20% of their initial body weight by week 28 (Weintraub, 1992). This reduction in body weight is considerably greater than the average weight loss obtained on single-dose regimens. Although the fenfluramine/phentermine combination will no longer be used clinically for the treatment of obesity, it has set a new 'gold standard' for antiobesity drugs. Future anorectics and/or combinations of anorectics should be developed that reduce body weight and food intake to the same extent (or greater) as fenfluramine/phentermine, but without the problematic side effects. Useful information in this pursuit can be gleaned by continuing research on the neurochemical mechanisms underlying fenfluramine/phentermine-induced hypophagia. As a first step in understanding *why* the fenfluramine/phentermine combination was so effective, we have begun to explore exactly *how* effective the fenfluramine/phentermine combination is. Specif-

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ically, when two anorectic drugs that target different neurotransmitter systems are co-administered, will they exert infra-additive (e.g., antagonistic), dose-additive or greater than additive (e.g., synergistic) anorectic effects?

One empirical model for determining drug synergy is to perform an isobolographic analysis. Isobolographic analyses were introduced by Loewe and Muischnek (1926) and have since been widely employed in the study of interactive effects of anticancer agents, investigations of lethal effects of combinations of analgesics and barbiturates, and interactions of α- and β-adrenoceptor agonists (Wessinger, 1986; Berenbaum, 1989). Thus, we have also been applying this type of analysis to examine interactive effects of drugs that target different neurotransmitter systems and have profound effects on feeding behavior.

In the following experiments, we systematically examined the efficacy of dexfenfluramine alone, phentermine alone and several dexfenfluramine/phentermine combinations for suppressing 90 min sweetened milk intake. These intake data were then plotted on isobolograms to determine whether dexfenfluramine and phentermine were interacting in an additive or synergistic manner. Because the anorectic effects of dexfenfluramine/phentermine have not been evaluated under deprived vs. nondeprived conditions and in light of previous reports that phentermine and other catecholaminergic receptor agonists may interact with deprivation state (Papasava et al., 1981, 1985; Caroll et al., 1981), we tested groups of rats under ad lib-fed conditions and following 24 h food deprivation.

2. Materials and methods

2.1. Animals and housing

Adult female (350–450 g) Sprague–Dawley rats ($n = 144$) were purchased as retired breeders (~ 6 months old) from Harlan (Indianapolis, IN). All rats were housed singly in suspended steel mesh cages in a vivarium maintained at 23 ± 1°C and illuminated from 0600–1800 h. Intake tests were performed during the daytime between 0900 and 1100 h. Unless otherwise specified, rats had ad lib access to Purina Chow pellets and tap water. All experiments were conducted in accordance with 'Principles of Laboratory Animal Care' set forth by NIH.

2.2. Drugs

Dexfenfluramine hydrochloride was a gift from Servier (Neuilly, France). Phentermine hydrochloride was purchased from Sigma (St. Louis, MO, USA). Doses are expressed as weight of the salts. Drugs were dissolved in saline and administered intraperitoneally 30 min prior to intake tests. When drug combinations were tested, they were mixed in the same solution and administered in the same injection volume as the individual drugs.

2.3. Statistical analysis

All statistics were conducted with SYSTAT for Windows software package. Data were first analyzed with analysis of variance (ANOVA) and post-hoc Bonferroni

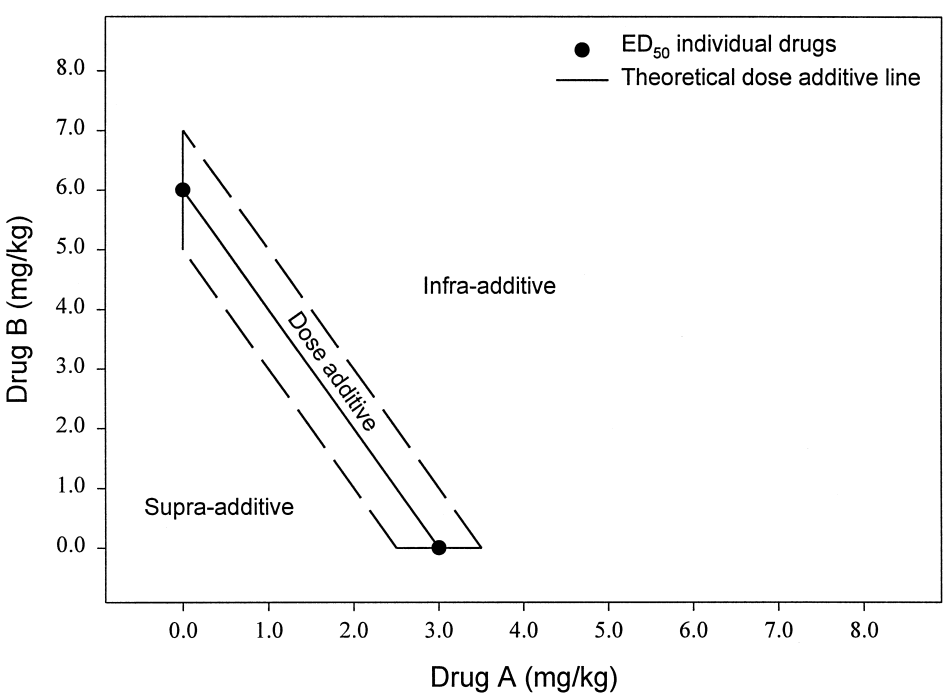

Fig. 1. Example of a hypothetical isobolographic plot. Filled circles represent equally effective doses of Drugs A and B; these points are plotted with their 95% confidence limits. The points and confidence limits are connected to yield a dose-additive 'region'. Equieffective doses of the drug combination that fall within this region are dose-additive, supra-additive below this region and infra-additive above this region.

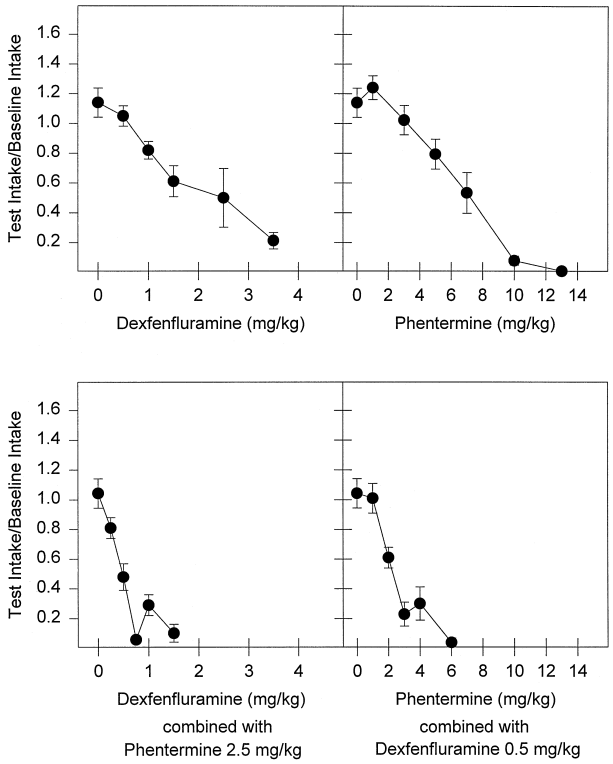

Fig. 2. Dose-response curves for dexfenfluramine, phentermine, a combination of a fixed dose of phentermine combined with a range of doses of dexfenfluramine and a combination of a fixed dose of dexfenfluramine combined with a range of doses of phentermine. Rats were nondeprived at time of testing. ED_{50} values for all curves were estimated using nonlinear regression.

comparisons were computed when appropriate. Significance level for all tests was set at $P < 0.05$. Next, the ED_{50} values and their 95% confidence intervals (CI) were calculated from the dose-response curves of the individual drugs and the combinations using nonlinear regression.

2.4. Isobolographic analysis

The mathematical validation and rationale behind isobolographic analyses have been extensively reviewed elsewhere (Wessinger, 1986; Berenbaum, 1989). The methodology of the analysis can briefly be described with the following hypothetical example. First, dose-response curves are generated for the individual drugs (e.g., drug A and drug B) and the ED_{50} (in our case, effective doses for reducing intake by 50%) values are computed using regression analysis. Next, a fixed dose of drug A is combined with a range of doses of drug B. Likewise, a fixed dose of drug B is combined with a range of doses of drug A. Again, using regression analysis, the ED_{50} values for the combinations are computed. The results from these experiments are then plotted on an isobologram (Fig. 1).

On the hypothetical isobologram, the *x*-axis contains increasing doses of drug A and the *y*-axis contains increasing doses of drug B. The ED_{50} values (and the 95% CI) for the individual drugs are plotted on the isobologram and then connected to form a 'theoretical' dose-additive line. The rationale behind this is to determine where the ED_{50} values of the drug combinations fall relative to the theoretical dose-additive line ($\pm$95% CI). If the ED_{50} values fall: (1) *above* these boundaries, then the combination is *infra-additive*, (2) *within* these boundaries, then the combination is *dose-additive* or (3) *below* the boundaries, then the combination is *supra-additive*.

2.5. Procedures

2.5.1. Experiment 1: Isobolographic analysis of the dexfenfluramine / phentermine combination on sweetened milk intake in nondeprived rats

Rats ($n = 70$) were adapted to drinking a sweetened milk solution (100 g commercial sugar and 100 g powdered milk per liter) in the home cage for 90 min a day until their intakes stabilized. Stabilization was defined as three consecutive days of consistent intake $\pm$2–3 ml. Once stable intakes were achieved (after 10 days), rats were divided into injection groups ($n = 5$–6 per group) that were matched for baseline intake. On test day (day 11), rats were injected with either saline, dexfenfluramine (0.5, 1.0, 1.5, 2.5 and 3.5 mg/kg) or phentermine (1.0, 3.0, 5.0, 7.0, 10.0 and 13.0 mg/kg). Thirty minutes later, sweetened milk solution was presented and total intake ($\pm$0.5 ml) was recorded after 90 min. On days 12–16, rats had daily access to sweetened milk, but did not receive any drugs. This time period enabled their intakes to restabilize. Rats were then divided into groups that were balanced as close as possible both for previous injection group and for their new baseline intakes. On day 17, rats were injected with one of the following drug combinations 30 min before testing: a fixed dose of phentermine (2.5 mg/kg) was combined with a range of doses of dexfenfluramine (0.25, 0.5, 0.75, 1.0 and 1.5 mg/kg), and a fixed dose of dexfenfluramine (0.5 mg/kg) was combined with a range

Table 1
Effects of dexfenfluramine and phentermine, alone and in combination, on 90 min sweetened milk intake in nondeprived rats

Drug and doses (mg/kg)	ANOVA	ED_{50} ($\pm$95% CI) [mg/kg]
dexfenfluramine (0.5–3.5)	$F(5,30) = 12.2$, $P < 0.01$	2.5 (1.3–3.6)
phentermine (1.0–13.0)	$F(6,35) = 30.4$, $P < 0.01$	6.3 (5.3–7.3)
phentermine (2.5) + dexfenfluramine (0.25–1.5)	$F(5,33) = 25.4$, $P < 0.01$	phentermine (2.5) + dexfenfluramine 0.4 (0.2–0.7)
dexfenfluramine (0.5) + phentermine (1.0–6.0)	$F(5,33) = 23.3$, $P < 0.01$	dexfenfluramine (0.5) + phentermine 2.3 (1.6–3.0)

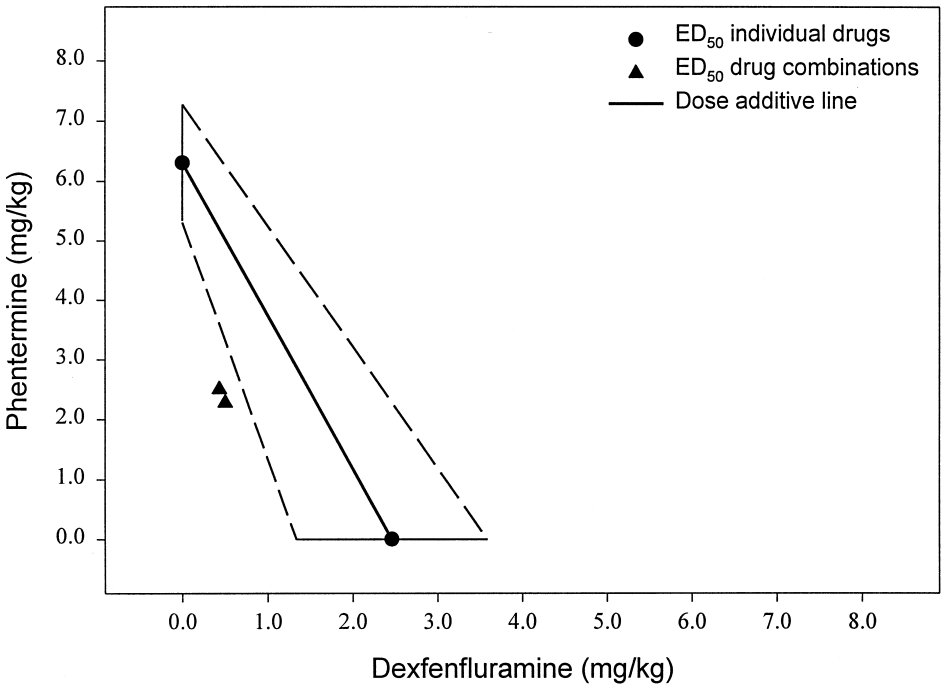

Fig. 3. Isobolographic plot for the anorectic effects of the dexfenfluramine/phentermine combination in nondeprived rats drinking sweetened milk. Notice that the doses of both of the combinations fall outside of the dose-additive region.

of doses of phentermine (1.0, 2.0, 3.0, 4.0 and 6.0 mg/kg). In sum, all rats were injected with drug and tested twice—once with an individual drug (on day 11) and once with a drug combination (on day 17).

2.5.2. Experiment 2: Isobolographic analysis of the dexfenfluramine / phentermine combination on sweetened milk intake in food-deprived rats

As in the previous experiment, new rats ($n = 72$) were adapted to drinking a sweetened milk solution (100 g commercial sugar and 100 g powdered milk per liter) in the home cage for 90 min a day. The major difference between these two experiments was that when rats were being habituated to consuming sweetened milk in Experiment 2, they were tested following 24 h food (but not water) deprivation on days 8, 11 and 14. The rationale was to obtain a stable baseline of food-deprived sweetened milk intake against which to measure the effects of the individual drugs and the combinations. Food-deprived milk intake on day 14 served as baseline intake for statistical comparisons. The days of nondeprived milk intake interspersed between the periods of 24 h food deprivation allowed for the rats' bodyweight to return to baseline. Thus, on day 17, 24 h food-deprived rats ($n = 5$–6 per group) were injected with either dexfenfluramine (0.5, 1.0, 1.5, 3.0 and 4.0 mg/kg) or phentermine (1.0, 3.0, 5.0, 7.0, 10.0 and 13.0 mg/kg). On day 20, 24 h food-deprived rats were injected with either a fixed dose of phentermine (2.5 mg/kg) combined with a range of doses of dexfenfluramine (0.5, 0.75, 1 and 1.5 mg/kg), or a fixed dose of

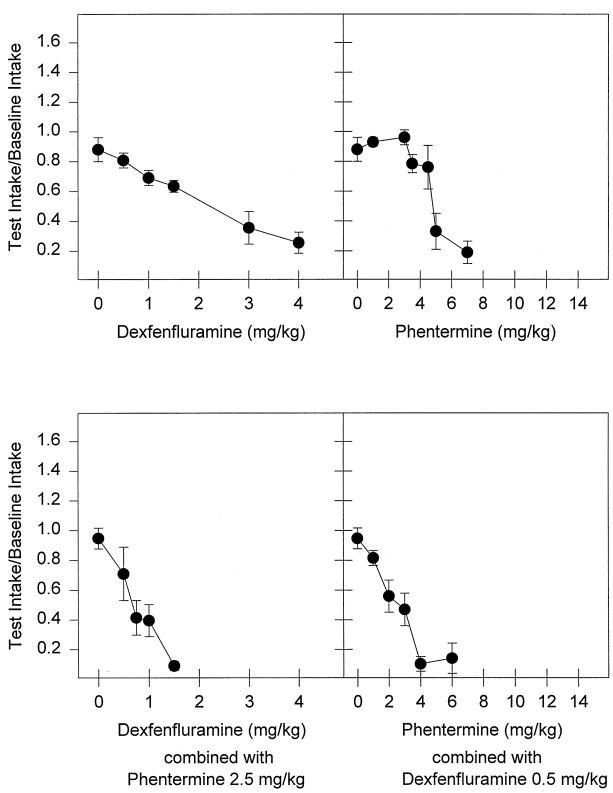

Fig. 4. Dose-response curves for dexfenfluramine, phentermine, a combination of a fixed dose of phentermine combined with a range of doses of dexfenfluramine and a combination of a fixed dose of dexfenfluramine combined with a range of doses of phentermine. Rats were 24 h food-deprived at time of testing. ED_{50} values for all curves were estimated using nonlinear regression.

Table 2
Effects of dexfenfluramine and phentermine, alone and in combination, on 90 min sweetened milk intake in 24 h food-deprived rats

Drug and doses (mg/kg)	ANOVA	ED_{50} (±95% CI) mg/kg
dexfenfluramine (0.5–4.0)	$F(5,29) = 11.7,\ P < 0.01$	2.4 (2.1–2.7)
phentermine (1.0–7.0)	$F(6,31) = 11.1,\ P < 0.01$	4.9 (4.1–5.7)
phentermine (2.5) + dexfenfluramine (0.75–1.5)	$F(4,26) = 8.9,\ P < 0.01$	phentermine (2.5) + dexfenfluramine 0.8 (0.5–1.2)
dexfenfluramine (0.5) + phentermine (1.0–6.0)	$F(5,34) = 15.4,\ P < 0.01$	dexfenfluramine (0.5) + phentermine 2.4 (1.5–3.3)

dexfenfluramine (0.5 mg/kg) combined with a range of doses of phentermine (1.0, 2.0, 3.0, 4.0 and 6.0 mg/kg).

3. Results

3.1. Experiment 1: Isobolographic analysis of the dexfenfluramine / phentermine combination on sweetened milk intake in nondeprived rats

Mean baseline intake was 21.3 ± 0.5 ml/90 min. Fig. 2 shows the dose-response curves for the anorectic effects of dexfenfluramine, phentermine and the dexfenfluramine/phentermine combinations on milk intake in nondeprived rats.

Intake data were analyzed as follows: (1) milk intakes were transformed by dividing test intake by baseline intake, (2) analysis of variance and post-hoc tests were performed on these scores, and (3) nonlinear regression was used to fit curves to the dose-response data to determine one-half maximum asymptote (ED_{50}), the asymptotic standard error and the 95% CI. These results (except asymptotic standard errors) are summarized in Table 1.

To determine whether dexfenfluramine and phentermine interacted in an additive or supra-additive manner, these results were plotted on an isobolographic plot. Drug interactions were considered to be significantly different from dose-additive if the ED_{50} values for the drug combinations did not overlap the 95% CI of the theoretical dose-additive line. Fig. 3 shows that dexfenfluramine and phentermine appear to interact in a supra-additive manner; the ED_{50} values for the two combinations both fall outside of the 95% CI of the dose-additive line.

3.2. Experiment 2: Isobolographic analysis of the dexfenfluramine / phentermine combination on sweetened milk intake in 24 h food-deprived rats

During adaptation, rats consumed significantly [$T(69) = 7.5,\ P < 0.01$] more milk following 24 h food deprivation ($x = 25.3$ ml/90 min) compared with under nondeprived conditions in the previous study ($x = 21.3$ ml/90 min).

Intake data were analyzed in an identical manner for Experiment 2. Fig. 4 shows the dose-response curves for

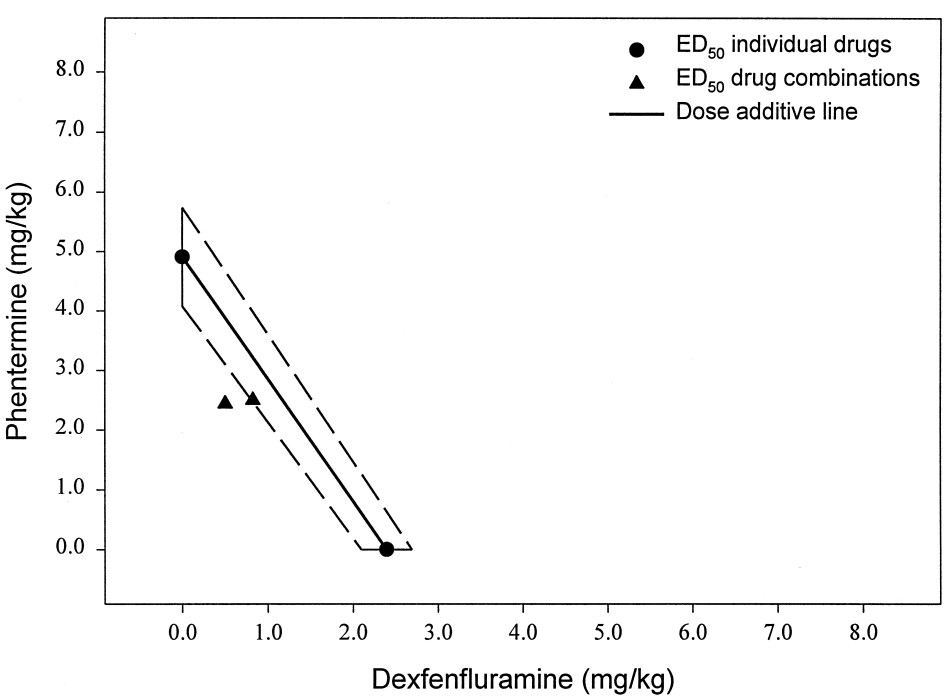

Fig. 5. Isobolographic plot for the effects of the dexfenfluramine/phentermine combination in 24 h food-deprived rats drinking sweetened milk. One combination fell outside of the dose-additive region, while the other combination fell just within the dose-additive region.

dexfenfluramine, phentermine and the dexfenfluramine/phentermine combinations in 24 h food-deprived rats. These results are summarized in Table 2.

When rats were tested following 24 h food deprivation, it required approximately 2.4 mg/kg of dexfenfluramine alone to suppress 90 min sweetened milk intake by 50%. When dexfenfluramine was combined with phentermine (2.5 mg/kg), the ED_{50} value was reduced to dexfenfluramine (0.8 mg/kg). These results are similar to those obtained when rats were tested under nondeprived conditions. In contrast, it required less phentermine to suppress 90 min sweetened milk intake in food-deprived rats (4.9 mg/kg) relative to nondeprived rats (6.3 mg/kg; Experiment 1). When a range of doses of phentermine were combined with a fixed dose of dexfenfluramine (0.5 mg/kg), the ED_{50} was reduced to phentermine (2.4 mg/kg). These ED_{50} values were also almost identical to those obtained in nondeprived rats; the dexfenfluramine/phentermine combination appears to be equipotent across deprivation state.

As in Experiment 1, these results were plotted on an isobologram (Fig. 5). In this case, the ED_{50} value for one of the drug combinations fell outside of the 95% CI of the dose-additive line, while the ED_{50} value for the other drug combination fell just within the 95% CI.

4. Discussion

Recently, we compared the anorectic efficacy of dexfenfluramine, phentermine and the dexfenfluramine/phentermine combination under acute and chronic conditions in multiple paradigms of stimulated intake (Roth and Rowland, 1998). In acute intake tests, combining a full dose of dexfenfluramine with a full dose of phentermine produced at least double the anorectic effect of the individual drugs. Additionally, it took a considerably longer period of time for the rats to develop anorectic tolerance to the dexfenfluramine/phentermine combination. In the present paper, we have extended these single-dose studies by analyzing the anorectic effects of a range of doses of the individual drugs and combinations using an isobolographic analysis.

When both drugs are capable of producing the effect under study (e.g., both dexfenfluramine and phentermine produce anorexia), single-dose studies provide limited information regarding the magnitude of a drug interaction. In other words, it becomes difficult to interpret the degree of the interaction as additive or synergistic because the expected outcome of the combination is *always* expected to exceed either of the individual drugs. The isobolographic analysis circumvents this difficulty by comparing equieffective doses of the individual drugs and the combinations; dose *and* effect are taken into account (Wessinger, 1986).

Do combinations of anorectic drugs that target different neurotransmitter systems exert additive or synergistic effects? The results from our isobolographic analysis suggest that, at least under acute conditions, dexfenfluramine and phentermine *do* exert synergistic anorectic effects. When these drugs were administered to nondeprived rats consuming a highly palatable milk solution, the ED_{50} values for both dexfenfluramine/phentermine combinations fell outside the 95% CI of the dose-additive line. Similarly, when rats were tested following 24 h food deprivation, the ED_{50} for one combination fell below the 95% CI of the dose-additive line and the other combination fell just within the boundaries of the 95% CI. The dexfenfluramine/phentermine combination produced anorectic effects that are greater than those predicted by their separate effects, at least at these doses.

Wellman et al. (1995) used an isobolographic analysis to examine the combined effects of fenfluramine (a serotonergic) and phenylpropanolamine (an α-1 adrenoceptor agonist) on 60 min food intake in 16 h food-deprived rats. Their results supported a dose-additive interaction between fenfluramine and phenylpropanolamine. This provides further evidence that simultaneously targeting multiple transmitter systems with anorectic drugs may be a promising approach and will probably yield at least dose-additive anorectic effects. The constituents of an ideal drug combination could conceivably be prescribed in low enough doses that would still yield powerful hypophagic effects, but have a lower incidence of adverse side effects relative to larger doses of individual drugs.

It is noteworthy that in the present paper and in similar publications by others (e.g., Foltin et al., 1983; Wellman et al., 1995), the ED_{50} values for the mixtures were obtained from dose-response curves that were generated by combining a fixed dose of one drug with a range of doses of the other drug. A more valid method for obtaining ED_{50} values for the combinations is for each point on the response curve to reflect a fixed ratio of drug A to drug B. For a more in-depth explanation on the use of fixed-ratio drug combinations in the study of drug interactions, we refer the reader to Tallarida and Raffa (1996). Future analyses on the interactive effects of anorectic agents should consider this statistical methodology.

In a pilot experiment on the effects of phentermine on 90 min chow intake in 24 h food-deprived rats, we calculated the ED_{50} for phentermine to be 3.3 mg/kg and noted that intake was suppressed by ~90% at 5 mg/kg (Roth and Rowland, unpublished). However, a dose of 5 mg/kg did not significantly attenuate 1 h sweetened milk intake in nondeprived rats (Roth and Rowland, 1998). Based on this, we hypothesized that phentermine might be a more potent anorectic under conditions of food deprivation. However, comparing chow intake in food-deprived rats to milk intake in nondeprived rats may be confounded because both deprivation state and the properties of the food are different. In the present experiments, we attempted to hold the food stimulus constant and test rats under deprived vs. nondeprived conditions. Ad lib-fed rats consumed ~21

ml/90 min, while 24 h food-deprived rats consumed ~25 ml/90 min. Because intake is spontaneous and robust under nondeprived conditions, and deprivation does not excessively elevate consumption, comparisons between nondeprived and deprived sweetened milk intake may prove useful in evaluating whether an agent interacts with deprivation state. In this case, deprivation state did not appear to have a major impact on the efficacy of the anorectics. The dose-response curves for dexfenfluramine and the dexfenfluramine/phentermine combinations were almost identical across deprivation state. There was a slight shift in the dose-response curve for phentermine, such that the $ED_{50} \pm 95\%$ CI value was 4.9 (4.1–5.7) in deprived rats vs. 6.3 (5.3–7.3) mg/kg in nondeprived rats. Phentermine appears to exert somewhat more effective anorectic effects under food-deprived conditions, although the 95% CI do overlap. Consistent with our findings, in self-administration studies (Papasava et al., 1981, 1985), the reinforcement efficacy of phentermine has also been shown to vary as a function of deprivation state. At 80% of free-feeding weight, rats will self-administer significant amounts of phentermine, while their free-feeding counterparts do not.

What are the neurochemical mechanisms that mediate the anorectic effects of the dexfenfluramine/phentermine combination? Dexfenfluramine is an indirect 5-HT agonist that promotes the release of 5-HT and prevents its reuptake (Samanin and Garratini, 1993). Dexfenfluramine is metabolized in vivo into a highly active metabolite, dexnorfenfluramine. Dexnorfenfluramine appears to have both direct agonist ($5\text{-}HT_{2C}$) and indirect effects on brain 5-HT systems (Curzon et al., 1997). In the present experiments, we used short-term (90 min) intake tests and most of the anorexia is probably attributable to dexfenfluramine's actions (although dexnorfenfluramine's effects were not ruled out). Nonetheless, it is important to point out that under conditions of long-term administration, it is likely that phentermine would interact both with dexnorfenfluramine *and* dexfenfluramine. Whether a combination of dexnorfenfluramine/phentermine (e.g., more of a direct 5-HT agonist relative to dexfenfluramine with a catecholaminergic) would be sufficient to yield synergistic anorectic effects remains to be examined.

Phentermine's anorectic effects are thought to be mediated by noradrenaline and dopamine. 6-Hydroxydopamine lesions have been shown to strongly attenuate the anorectic effects of lower doses of phentermine (2.5–5.0 mg/kg), but they do not reduce the anorectic effects of higher doses (10 mg/kg; Samanin et al., 1975). One explanation may be that high doses of phentermine also elevate levels of brain 5-HT and this is enough to support phentermine's anorectic effects. Indeed, recent studies using in vivo microdialysis suggest that phentermine enhances extracellular levels of dopamine *and* 5-HT in the nucleus accumbens (Shoaib et al., 1997), but only dopamine in the striatum (Balcioglu and Wurtman, 1998). In the present experiments, a potential neurochemical correlate for dexfenfluramine/phentermine's anorectic effects may involve enhanced brain 5-HT levels.

5. Conclusion

In theory, the use of combinations of anorectic drugs appears to be quite attractive. In the present paper, we have shown that under acute conditions, dexfenfluramine and phentermine will interact in a synergistic manner to suppress milk intake in nondeprived and 24 h food-deprived rats. We have also shown that under chronic conditions, dexfenfluramine/phentermine-treated rats lose far more weight than rats treated with either drug alone (Roth and Rowland, 1998). The effectiveness of simultaneously targeting multiple systems has also been demonstrated by Wellman et al. (1995) on the combination of fenfluramine and phenylpropanolamine, and by Jackson et al. (1997) on the serotonin–noradrenaline reuptake inhibitor sibutramine. However, the clinical use of anorectic drugs has a very 'checkered' past (Bray, 1997): amphetamines have been associated with drug addiction, aminorex and fenfluramine with pulmonary hypertension, and of course, the recent problems of valvular insufficiency associated with fenfluramine and fenfluramine/phentermine. This suggests that (1) the risk–benefit equation with respect to the clinical use of anorectics needs to be re-evaluated, and (2) if combining diet drugs is to play an important role in obesity management, more research on their safety and efficacy is warranted.

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