Effects of a Cannabinoid$_1$ Receptor Antagonist and Serotonin$_{2C}$ Receptor Agonist Alone and in Combination on Motivation for Palatable Food: A Dose-Addition Analysis Study in Mice

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ABSTRACT

The cannabinoid and serotonin systems modulate feeding behavior in humans and laboratory animals. The present study assessed whether a cannabinoid (CB)$_1$ receptor antagonist and a serotonin (5-HT)$_{2C}$ receptor agonist alone and in combination attenuate motivation for the liquid nutritional drink Ensure as measured by a progressive ratio (PR) schedule of reinforcement in male C57BL/6 mice. Pretreatment (15 min i.p.) with either the CB$_1$ receptor antagonist $N$-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboximide hydrochloride (SR141716) (SR; Rimonabant or Acomplia) or the 5-HT$_{2C}$ receptor agonist $m$-chlorophenylpiperazine (mCPP) dose-dependently decreased the maximum ratio completed under the PR schedule (break point) in mice. ED$_{25}$ values for SR and mCPP to decrease break point were determined, and the relative potency of each drug alone was quantified. Fixed dose-ratio pairs of SR/mCPP based on their relative potency were then administered. Dose-addition analysis comparing the experimentally determined potency for SR/mCPP combinations with their predicted additive potency revealed that SR/mCPP combinations in 1:1 and 2:1 ratios based on relative potency produced significant synergistic attenuation of break point for Ensure. The ED$_{25}$ values for decreasing break point were consistently lower than ED$_{25}$ values for decreasing response rate, and synergistic effects of SR/mCPP combinations on break point were seen independent of synergistic effects on response rate. These results indicate that cannabinoid CB$_1$ and serotonin 5-HT$_{2C}$ receptors are involved in motivated feeding behavior in mice and that these compounds can synergistically modulate motivation for palatable food with the synergy dependent upon the ratio of SR/mCPP in the combination.

The dysregulation of neural systems underlying ingestive behavior can contribute to excessive eating and obesity. Two neural systems currently being targeted to correct this dysregulation are the cannabinoid (CB) and serotonin (5-HT) systems.

The appetite-stimulating effects of Cannabis sativa (marijuana) have been known for almost 2000 years. The major active constituent of marijuana, $\Delta^9$-tetrahydrocannabinol, and other CB receptor agonists are associated with excessive hunger and overeating in humans and animals (for review, see Berry and Mechoulam, 2002), and CB receptor agonism is currently used for appetite stimulation in human immunodeficiency virus/acquired immunodeficiency syndrome patients. These actions seem to be mediated by cannabinoid CB$_1$ subtype receptors. CB$_1$ receptor knockout mice show decreased food intake (Poncelet et al., 2003; Ravinet Trillou, 2004) as well as decreased self-administration of palatable food (Sanchis-Segura et al., 2004; Ward and Dykstra, 2005), and selective CB$_1$ receptor antagonists, such as SR141716 (Rimonabant or Acomplia), decrease food intake (Colombo et al., 1998; Wiley et al. 2005) and self-administration of palatable food (Arnone et al., 1997; Ward and Dykstra, 2005) in laboratory animals. SR141716 produces sustained weight loss in human subjects (Van Gaal et al., 2005) and is available under prescription in several EU countries; SR141716 was recently rejected by the U.S. Food and Drug Adminis-
tration due to the risk of increased psychiatric adverse events, including depressed mood and suicidality at the therapeutic doses used to produce moderate levels of weight loss (Traynor, 2007).

The 5-HT receptor system also plays a well established role in appetite regulation, with 5-HT_{2C} receptors being perhaps the most widely implicated receptor subtype (for review, see Halford et al., 2007). For example, mice lacking the 5-HT_{2C} receptor are hyperphagic and develop late-onset obesity (Tesco et al., 1995), whereas agonists with activity at the 5-HT_{2C} receptor produce well documented decreases in ingestive behavior. The 5-HT_{2C} receptor agonist m-chlorophenylpiperazine (mCPP) decreases food intake and produces chronic reductions in body weight in laboratory animals (Samanin et al., 1979; Hewitt et al., 2002) and has been demonstrated to decrease self-administration of food pellets in pigeons (Wolff and Leander, 2000). In humans, the 5-HT indirect agonists fenfluramine and dexfenfluramine, used for several years to treat obesity, produce significant, sustained decrease in appetite and body weight (Finer et al., 1987) that have been attributed to its high affinity for 5-HT_{2C} receptors (Curzon et al., 1997). mCPP has also been tested in humans as a potential antiobesity agent; however, at doses that decreased appetite, mCPP was also reported to produce anxiety (Coven et al., 1995).

Although neurochemical evidence suggests that the cannabinoid receptor system can modulate serotonin activity in the brain (Tzavara et al., 2003), to the authors' knowledge, only one study to date has assessed whether CB1 antagonist/5-HT agonist combinations produce synergistic effects on ingestive behavior. Rowland et al. (2001) coadministered SR141716 and dexfenfluramine in rats with free access to food in their home cage; the combined effect of these compounds produced an additive reduction in sweet milk consumption. However, the regulation of feeding is complex and includes control of both consummatory as well as appetitive aspects of ingestive behavior, and whether CB1 antagonist/5-HT agonist combinations can synergistically alter motivation to seek a food reinforcer is unknown. The identification of drug combinations that synergistically attenuate motivation for palatable foods in preclinical models can contribute to the identification of promising antiobesity combination pharmacotherapies because the limited number of single antiobesity agents currently available suffer from low clinical efficacy (Bray and Greenway, 2007).

The purpose of the present study was to determine whether the CB1 antagonist SR141716 and the 5-HT_{2C} agonist mCPP can act synergistically to decrease motivation to consume a palatable food in a mouse model of motivated behavior. First, we assessed the effect of SR141716 or mCPP alone on operant responding for the liquid nutritional drink Ensure under a progressive ratio (PR) schedule of reinforcement. Second, we assessed the effect of combinations of different proportions of SR141716 and mCPP on responding for Ensure under the PR schedule. Dose-addition and isobolographic analyses were used to determine whether these compounds produce additive, subadditive, or synergistic effects on motivation to consume a palatable food. The effect of SR141716 and mCPP alone and in combination on the rate of responding during PR sessions was also measured and analyzed to investigate whether potential synergistic effects on PR performance could be accounted for by a magnification of rate-decreasing effects of the drugs as opposed to a more selective effect on motivation.

Materials and Methods

Subjects

Male Swiss-Webster mice (n = 12) obtained from ACE Animals Inc. (Boyertown, PA) weighing 30 to 40 g were used. The mice lived in a humidity- and temperature-controlled room under a 12-h light/ dark schedule (lights on, 7:00 AM). The mice received water ad libitum and 3 g of food per day after each experimental session. Vehicle tests occurred four to five times per week, drug tests occurred one to two times per week, and experimental sessions began at approximately 10:00 AM. All experimental procedures strictly conformed to the guidelines of the Institutional Animal Care and Use Committee of Temple University and with the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the U.S. National Institutes of Health.

Apparatus

Experiments were conducted in mouse experimental conditioning chambers (21.6 \( \times \) 17.8 \( \times \) 12.7 cm, model ENV-307W, MED Associates, St. Albans, VT) located within ventilated sound-attenuating cubicles. The operant conditioning chambers were equipped with two nose-poke holes (1.2-cm diameter) containing internal stimulus lights and a motor-driven dipper for liquid presentation. The receptacle opening for access to the dipper was located between the two nose-poke holes and also contained an internal stimulus light. The chambers were also equipped with a house light and ventilator fan.

Behavioral Training: PR Responding for Ensure

Mice were first trained to nose-poke into the illuminated (active) nose-poke hole under a fixed ratio (FR) 1 schedule wherein each correct nose-poke resulted in the delivery of 0.01 cc vanilla-flavored liquid nutritional drink, Ensure Plus, for 3 s. During the 3-s food delivery, the house light and active nose-poke hole light were turned off, and the food receptacle was illuminated.

After achieving stable levels of responding under the FR1 schedule, mice were trained to respond for Ensure under a PR schedule of reinforcement, wherein the ratio requirement to obtain a reinforcer increases throughout a test session. The break point is defined as the largest ratio completed in the session. Performance under PR schedules of food delivery is considered to reflect the efficacy or motivational strength of food, because increases in either deprivation level or reinforcer magnitude increase break points (Hodos and Kalman, 1963). The following modified log progression of response requirements was used in the present study: 1, 2, 4, 6, 9, 12, 15, 20, 25, 32, 40, 50, 62, 77, 95, 118, 145, etc. Completion of the ratio resulted in the delivery of 0.01 cc Ensure Plus for 3 s. During food delivery, the house light and active nose-poke hole light were turned off, and the food receptacle was illuminated.

Break point was defined as the final ratio completed by the end of the 1-h test session or before 10 min elapsed without a reinforcer earned. This progression of response requirements coupled with the time constraints was chosen because vehicle-treated animals routinely achieved a break point within 20 to 30 min, well within the 1-h session time constraint. Using such a protocol increases experimenters' ability to parse out effects on motivation versus overall suppression of response rate produced by a pharmacological manipulation, which is a central feature of PR schedules of reinforcement (for review, see Stafford et al., 1998).

Effect of SR141716 and mCPP Alone on PR Responding

Stable responding under the PR schedule was defined as 2 days of baseline responding, where the number of reinforcers earned differed by no more than three. Once stable responding was achieved, the effect of the CB1 antagonist SR141716 on break point was as-
sessed. Mice were pretreated with vehicle before baseline sessions and pretreated with a dose of SR141716 (0.3–30 mg/kg) in a randomized design before the test sessions. Each test followed at least 2 days of stable baseline responding and occurred once or twice per week (see Fig. 1A).

Vehicle or drug was injected i.p. 15 min before the 1-h sessions. The effect of each SR141716 dose on break point was determined in at least eight of the 12 mice in the study, depending on the number of mice responding stably before the test days.

After determination of the SR141716 dose-effect curve, the same mice were used to assess the effect of the 5-HT₂C agonist mCPP (0.1–10 mg/kg mCPP) on break point under identical conditions. In addition, doses of each drug that decreased break point by 25% (ED₂₅ values) were readministered in these animals midway through the SR/mCPP combination testing to determine whether tolerance or sensitization to the effect of SR or mCPP occurred during the study.

Effect of SR/mCPP Combinations on PR Responding

SR/mCPP combinations will be described in two ways throughout the manuscript. SR/mCPP combinations will be primarily referred to by the ratio of the two drugs administered based on their relative potencies. For example, a combination dose given as a 1:1 ratio of SR/mCPP based on their relative potencies indicates that the combination is comprised of doses of each drug that produce identical effect levels, although the actual doses of each drug administered may differ. Therefore, the actual proportion of the two drugs that comprise various SR/mCPP ratios will also be reported. The proportion represents the quantity of each drug (milligrams per milliliter) present in 1 mg/ml of the combination.

The same mice used in the single agent studies were used to generate the experimentally determined dose-response curves for combinations of SR/mCPP under identical conditions, except that mice received two simultaneous vehicle injections on baseline days and two simultaneous drug injections on test days, in opposite sides of the peritoneal cavity. As in the single-dose studies described above, SR/mCPP combinations were also administered in a randomized design. ED₂₅ values of each drug alone were used to establish the relative potency of the two compounds to decrease break point, and this relative potency was used to first test a series of SR/mCPP fixed dose-ratio pairs in a 1:1 ratio to generate a SR/mCPP dose-response curve. Based on these results, four additional dose-response curves were generated using fixed dose-ratio pairs comprised of other ratios of SR/mCPP based on their relative potency (3:1, 2:1, 1:2, 1:3 SR/mCPP) to test whether a combined synergistic effect depends upon the proportion of each drug in the combination because deviation from additivity can depend upon the relative proportions of the individual drugs under study (Tallarida, 2000; Fischer and Dykstra, 2006).

Data Analysis

Effect of SR141716 and mCPP Alone on PR Responding. To determine whether individual doses of SR and mCPP alone significantly attenuated break point and response rate, one-way repeated measures analyses of variance were performed (Prism version 4; GraphPad Software Inc., San Diego, CA). To determine ED₂₅ values for SR and mCPP, break points were transformed to a percentage change in the break point on test day by comparing the test day break point with the previous day’s baseline break point; therefore, baseline vehicle values for each dose were always determined the day before drug test to establish the most relevant control value, with each mouse serving as its own control (see Fig. 1A). The percentage of change in break point for individual mice produced by each dose of SR141716 or mCPP was determined using the following equation:

\[ 100 - \left(\frac{\text{test day break point}}{\text{previous vehicle day break point}}\right) \times 100 \] (1)

Individual percentage of changes in break point data were then averaged for each dose, and these dose-effect curves were used to determine the dose of each drug producing a 25% decrease (ED₂₅) in break point. ED₂₅s were derived by linear regression analysis, the relative potency (ED₂₅ less potent compound/ED₂₅ more potent compound) of SR/mCPP was established, and a theoretical predicted additive dose-response curve for equipotent dose-pairs of SR141716 and mCPP (SR/mCPP 1:1) was generated (PharmTools Pro 1.1; McCary Group, Inc. Elkins Park, PA). As a result, additional predicted additive dose-response curves were generated for fixed dose-ratio pairs of SR/mCPP in a 3:1, 2:1, 1:2, or 1:3 ratio based on their relative potencies. Linear regression analysis was used to test whether the

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**Fig. 1.** Assessment of the effect of mCPP or SR141716 on responding for Ensure under a PR schedule of reinforcement. A, schematic representation of the general order of vehicle and drug administration (for both single agent and combination dosing) throughout the study. Animals were administered either vehicle or drug 15 min before each behavioral session. Break point data from each vehicle control value session served as the baseline value for the following day’s drug test. Individual animals were tested on the drug test day only after having a stable response during the prior 2 vehicle days. B, effect of mCPP on change in break point (dark-gray squares) and response rate (light-gray squares) in mice responding for Ensure under a PR schedule of reinforcement. Abaris, dose of drug in milligrams per kilogram. Ordinates, mean percentage of decrease from baseline responding. Each point shows the mean (±S.E.M.) from eight to 12 mice.
effect of mCPP versus SR141716 on break point was significantly different [analysis of covariance (ANCOVA) test for equal Y intercepts; GraphPad Prism version 4].

The effect of SR141716 or mCPP on rate of responding during PR sessions was also analyzed. Rate of responding was calculated as the total number of active nose-pokes made divided by the total number of seconds during the experimental session and was expressed as the number of responses per second. The ratio of responding was converted to a percentage of decrease in response rate relative to baseline vehicle response rates by the following equation:

\[
100 - ((\text{test day rate}/\text{previous vehicle day rate}) \times 100)
\]  
(2)

Dose-effect curves for single agents were used to determine the dose of each drug required to produce a 25% decrease (ED\text{25}) in response rate. The relative potency of SR and mCPP to decrease response rate was calculated and used to generate theoretical predicted additive dose-response curves for the effects of the five SR/mCPP ratio combinations (1:1, 2:1, 3:1, 1:2, 1:3 SR/mCPP) on response rate. Linear regression analysis was used to test whether the effect of mCPP versus SR141716 on response rate was significantly different (ANCOVA test for equal y intercepts; GraphPad Prism 4).

The relative potencies of mCPP or SR141716 to decrease break point versus response rate were also calculated, and linear regression analysis was used to test whether the effect of each compound on break point was significantly different from its effect on response rate (ANCOVA test for equal y intercepts; GraphPad Prism 4).

**Effect of SR/mCPP Combinations on PR Responding.** Raw data from the five SR/mCPP experiments were transformed in an identical manner to the single agent data to establish five SR/mCPP dose-effect curves and generate ED\text{25} values for each. To determine the extent to which interactions between SR141716 and mCPP resulted in effects on break point and response rate that differed from dose additivity, both graphical and statistical analyses were performed (Tallarida, 2000, 2006). Dose additivity is based on the concept of dose equivalence, that is, that each drug in a combination contributes based on the individual drug potencies. Using the graphical approach, the distinctions among subadditive, additive, or synergistic interactions were made with the use of isobolograms. Isobolograms for both break point and response rate were constructed by connecting the ED\text{25} of SR141716 alone plotted on the abscissa with the ED\text{25} of mCPP alone plotted on the ordinate to obtain a line of additivity. The 25% effect level was selected because it is a magnitude achieved by both individual agents to decrease break point. The line of additivity contains the loci of dose pairs that produce an ED\text{25} equal to the ED\text{25} of SR141716 or mCPP alone. Dose pairs that fall to the left of the additivity line demonstrate that an ED\text{25} was reached with lesser quantities of the drugs, suggestive of synergism. In contrast, experimental points representing dose pairs that fall along the line are suggestive of additivity, and experimental points that fall to the right of the line are suggestive of subadditivity.

Drug interactions for each fixed dose-ratio combination were statistically analyzed by comparing the experimentally determined ED\text{25} values (Z\text{add}) to the predicted additive ED\text{25} values (Z\text{add}). The test for significance for the difference between Z\text{add} and Z\text{max} is based on Student’s t distribution (for full details, see Tallarida, 2000). In brief, because the sample means are derived from curve-fitting procedures and not from enumerated data, the modified t, designated t’, is determined as follows (Tallarida, 2000):

\[
 t' = \frac{Z\text{add} - Z\text{mix}}{[(S.E.\cdot Z_{\text{add}})^2 + (S.E.\cdot Z_{\text{mix}})^2]^{\frac{1}{2}}}
\]  
(3)

The t’ value is then compared with a computed t value, designated T, derived from table values t\text{c} and t\text{s}, as follows:

\[
 T = [t\text{c}(S.E.\cdot Z_{\text{add}})^2 + t\text{s}(S.E.\cdot Z_{\text{mix}})^2]/[(S.E.\cdot Z_{\text{add}})^2 + (S.E.\cdot Z_{\text{mix}})^2]
\]  
(4)

where \( t_c \) is the tabular value of \( t \) based on the sample size of \( Z_{\text{add}} - 2 \) df, and \( t_s \) is the tabular value of \( t \) based on the sample size of \( Z_{\text{mix}} - 2 \) df. If \( T > t \), the difference is significant.

The proportions of each drug used to generate the five predicted additive and experimentally determined SR/mCPP dose-response curves in the 1:1, 2:1, 3:1, 1:2, and 1:3 SR/mCPP ratios were based on the relative potency of SR/mCPP to decrease break point; hence, these ratios pertain specifically to the two drugs’ relationship regarding decreases in break point. All drug combination data in the text, figures, and tables are primarily labeled according to these ratios for consistency. However, because the relative potency of the drug to decrease response rates probably differs from their relative potency to decrease break point, the proportions of each drug in the combinations administered will reflect unique ratios based on the relative potency of SR and mCPP to decrease response rate. These unique ratios of SR/mCPP based on relative potency to decrease response rates for the five SR/mCPP combinations tested are reported under Results. In addition, relative potency to decrease response rate rather than break point were necessarily used when constructing the response rate isobolograms and for statistical analysis of combination response rate data.

The relative potencies of SR/mCPP combinations to decrease break point versus response rate were calculated and linear regression analysis was used to test whether the effect of SR/mCPP combinations on break point was significantly different from their effect on response rate (ANCOVA test for equal y intercepts; GraphPad Prism 4).

**Drugs**

SR141716A (Research Triangle Institute, Research Triangle Park, NC) was dissolved in a vehicle of 100% ethanol, Cremophor (Sigma-Aldrich, St. Louis MO), and saline in a ratio of 1:1:18. mCPP hydrochloride (Sigma-Aldrich) was dissolved in saline. Drugs were injected i.p. at a volume of 0.1 ml/10 g 15 min before behavioral testing. Drug doses are expressed as total weight of the salt (mCPP) or base (SR141716).

**Results**

**Effect of SR141716 and mCPP Alone on PR Responding.** Mice reached stable levels of progressive ratio responding in approximately 3 weeks from the start of FR1 training. After vehicle injections, representative baseline break points across the 12 mice ranged from between 32 responses (corresponding to 10 reinforcers earned) to 219 responses (corresponding to 19 reinforcers earned) (data not shown). Break points fluctuated slightly in individual mice throughout the series of experiments; however, each mouse was only administered drug on a test day if that individual had been exhibiting stable rates of baseline responding before the test day (see Materials and Methods). Figure 1 illustrates a schematic of the general order of vehicle and drug administration (for both single-agent and combination dosing) (1A) and the effects of mCPP (1B) and SR141716 (1C) alone on responding for Ensure under a PR schedule of reinforcement. Both SR141716 and mCPP produced dose-dependent decreases in break point and rate of responding. Repeated measures one-way analysis of variance revealed a statistically significant effect of SR on break point (\( F_{6,72} = 2.6, p < 0.05 \)) and response rate (\( F_{6,72} = 2.9, p < 0.05 \)) and of mCPP on break point (\( F_{5,59} = 10.9, p < 0.01 \)) and response rate (\( F_{5,59} = 5.2, p < 0.01 \)). For mCPP, the ED\text{25} value for decreasing break point was 0.300 ± 0.132, and the ED\text{25} value for decreasing response rate was 2.13 ± 1.27. mCPP was approximately 6.8 times more potent at decreasing break point than response
rate; however, the lines were not significantly different from one another \((F_{1,7} = 3.5, \text{N.S.})\). For SR141716, the ED\(_{25}\) value for decreasing break point was 2.29 \(\pm\) 0.611, and the ED\(_{25}\) value for decreasing responses rate was 5.53 \(\pm\) 1.60; therefore, SR141716 was approximately 2.4 times more potent at decreasing break point than response rate \((F_{1,9} = 4.9, p < 0.05)\). These ED\(_{25}\) values for break point were also administered alone midway through the combination experiments to verify that neither tolerance nor sensitization developed to either compound alone. After 16 weeks of single agent and combination testing twice weekly, administration of the ED\(_{25}\) value 0.3 mg/kg mCPP decreased break point responding by 29\%, and administration of the ED\(_{25}\) value 2.29 mg/kg SR decreased break point responding by 23\%. It is not possible to statistically compare effect of 2.29 mg/kg SR experimentally obtained at week 16 with the ED\(_{25}\) effect of 2.29 mg/kg SR derived from the initial SR dose-response curve; however, a 23\% reduction is probably not different from a 25\% reduction in a behaviorally meaningful way. It is a coincidence that the derived ED\(_{25}\) for mCPP (0.3 mg/kg) was also a dose that was experimentally tested during the initial mCPP dose-response curve, and the initial effect of 0.3 mg/kg mCPP was not statistically different from the week 16 effect of mCPP as indicated by Student’s \(t\) test \((p = 0.90)\). Therefore, the weekly test injections of SR, mCPP, or SR/mCPP combinations did not seem to produce tolerance or sensitization to the effects of SR or mCPP.

MCPP was approximately 7.6 times more potent than SR141716 at decreasing break point \((F_{1,8} = 21.1, p < 0.01)\) (Fig. 1), and the relative potency of SR141716 to mCPP was used to determine the relative proportions of the single agents required in each of the five fixed dose-ratio combinations to be tested. The actual proportions of each drug required to achieve the fixed dose-ratio combinations were determined to be the following: SR/mCPP given in a 1:1 ratio based on potency – proportion 0.88 SR/0.12 mCPP, SR/ mCPP given in a 2:1 ratio based on potency – proportion 0.94 SR/0.06 mCPP, SR/mCPP given in a 3:1 ratio based on potency – proportion 0.96 SR/0.04 mCPP, SR/mCPP given in a 1:2 ratio based on potency – proportion 0.79 SR/0.21 mCPP, and SR/mCPP given in a 1:3 ratio based on potency – proportion 0.72 SR/0.28 mCPP. The slope of the regression lines for SR141716 and mCPP to decrease breakpoint did not differ significantly, and linear isobolographic and statistical analyses were therefore conducted.

MCPP was approximately 2.6 times more potent than SR141716 at decreasing response rate, although this shift was not statistically significant. Therefore, the proportions of SR/mCPP tested in the five combinations result in the following ratios based on relative potency to decrease response rate: 0.88 SR/0.12 mCPP – 2.8:1 SR/mCPP, 0.94 SR/0.06 mCPP – 6:1:1 SR/mCPP, 0.96 SR/0.04 mCPP – 9:1 SR/ mCPP, 0.79 SR/0.21 mCPP – 1:5:1 SR/mCPP, and 0.72 SR/ 0.28 mCPP – 1:1 SR/mCPP. These ratios based on relative potency to decrease response rate are indicated alongside break point ratios when necessary for clarity.

**Effect of SR/mCPP Combinations on PR Responding.** Each drug combination produced dose-dependent decreases in break point and response rate (Fig. 2). Similar to the single agent data, drug combinations decreased break points at lower doses than they decreased response rates. For example, a 3.2-fold shift was observed for the 1:1 SR/mCPP combination \((F_{1,9} = 6.7, p < 0.05)\), 5.8-fold for the 2:1 combination \((F_{1,9} = 7.5, p < 0.05)\), 1.4-fold for the 3:1 combination \((F_{1,7} = 2.6, \text{N.S.})\), 3.1-fold for the 1:2 combination \((F_{1,6} = 6.6, p < 0.05)\), and 2.0-fold for the 1:3 combination \((F_{1,8} = 4.6, \text{N.S.})\). Isobolographic analyses (Fig. 3) illustrate that several combinations produced a decrease in break point that fell to the left of the predicted line of additivity, suggesting synergetic interactions. Combinations with the relatively equal ratio of SR141716 to mCPP based on potency produced ED\(_{25}\) values that fell to the left of the predicted line of additivity, and dose-addition statistical analysis revealed that the experimentally determined ED\(_{25}\) values for the 1:1 and 2:1 SR/mCPP combinations were significantly lower than the predicted additive ED\(_{25}\) values. In contrast, a high ratio of mCPP to SR (1:3 SR/mCPP) produced an ED\(_{25}\) value that fell to the right of the predicted line of additivity; the experimentally determined ED\(_{25}\) values for the 1:3, 1:2, and 3:1 SR/ mCPP combinations were not significantly different from the predicted ED\(_{25}\) values (Table 1).

Isobolographic analyses (Fig. 4) illustrate that all combinations produced a decrease in response rate that fell to the left of the predicted line of additivity, suggesting synergistic interactions on this measure as well. Dose-addition statistical analysis revealed that the experimentally determined ED\(_{25}\) values for the 1:1 or 1:2 combination ratios based on break point also produced a significant synergistic decrease in response rate, whereas the experimentally determined ED\(_{25}\) values for the other ratios were not significantly different from the predicted ED\(_{25}\) values (Table 2). As is depicted in the response rate isobolograms and stated above, one SR/mCPP proportion resulted in a ratio of SR/mCPP of 1:1 based on relative potency to decrease response rate (i.e., 0.72 SR/0.28 mCPP, compared with 0.88 SR/0.12 mCPP, which results in a 1:1 ratio based on break point). All other proportions tested were comprised of a larger ratio of SR/mCPP based on rate. The ratios at which significant synergistic effects on decrease in response rate occurred are 2.8:1 and 1.5:1 SR/mCPP based on relative potency to decrease response rate.

**Discussion**

The CB\(_1\) antagonist SR141716 and the 5-HT\(_{2C}\) agonist mCPP significantly and dose-dependently decreased the reinforcing efficacy of Ensure as measured by responding under a PR schedule. These data suggest that both drugs decrease motivation for Ensure, with mCPP approximately 8-fold more potent than SR141716. These results support growing evidence that CB\(_1\) antagonism decreases motivation as measured by PR schedules in laboratory animals for palatable food reinforcers, e.g., sucrose in rats (Economidou et al., 2006) and Ensure and corn oil in mice (Ward and Dyskstra, 2005). In addition, SR141716 decreases PR responding for several drug reinforcers, including cocaine, heroin, ethanol, and nicotine (Cohen et al., 2002; Solinas et al., 2003; Soria et al., 2005; Economidou et al., 2006, respectively), which taken together suggest that the cannabinoid receptor system positively modulates food and drug reinforcement. Although the hypophagic effects of mCPP have been well demonstrated, this is the first report to the authors’ knowledge to characterize in rodents the ability of 5-HT\(_{2C}\) receptor activation to attenuate the reinforcing efficacy of a palatable food.
food as measured by responding under a PR schedule. As previously mentioned, mCPP decreased PR responding for food pellets in pigeons (Wolff and Leander, 2000). Depletion of central 5-HT has been shown to significantly increase PR responding maintained by food or by cocaine (Roberts et al., 1994), and activation of 5-HT receptors decreases cocaine self-administration under a PR schedule (Richardson and Roberts, 1991; Grottick et al., 2000), suggesting that the 5-HT system is a negative modulator of food and drug reinforcement.

Isobolographic and dose-addition analyses revealed that coadministration of SR and mCPP can produce a synergistic attenuation of PR responding for Ensure. Five different ratio combinations were studied because deviation from additivity can depend upon the relative proportions of the individual drugs under study (Tallarida, 2000; Fischer and Dykstra, 2006). In agreement with these findings, the nature of the SR/mCPP interactions was dependent on the ratio of SR/mCPP administered. SR/mCPP combinations in a 3:1, 2:1, 1:1, or 1:2 ratio produced ED<sub>25</sub> values that fell to the left of the predicted line of additivity, suggesting synergism at these ratios. Statistically significant synergistic attenuation of PR responding occurred at SR/mCPP ratios of 1:1 and 2:1. SR/mCPP in a 3:1 or 1:2 ratio approached the line of additivity, whereas the 1:3 ratio produced an ED<sub>25</sub> value that fell to the right of the line of predicted additivity, approaching subadditivity. SR141716 and mCPP also decreased response rate in the present set of experiments; therefore, the contribution of nonspecific effects on response behavior such as suppressed locomotion and sedation cannot presently be ruled out for single agents and combinations.

MCPP has known sedative and hypolocomotive effects (Stiedl et al., 2007); in contrast, SR141716 does not seem to decrease locomotor activity or produce marked sedation (Tzavaras et al., 2003; Kelsey and Calabro, 2007; S.J. Ward, unpublished data) or may do so only at higher concentrations (Järbe et al., 2002). Both mCPP and SR were less potent at decreasing response rates than attenuating break point in the present study, suggesting that for both compounds, rate-decreasing effects are not necessary for a decrease in break point. Indeed, the decreases in response rates observed in the present set of experiments, especially by SR, may at least in part reflect increases in satiety and/or alteration in the perceived palatability of Ensure as opposed to gross motor impairment. SR/mCPP combinations also decreased response rate; however, regression analyses also revealed a shift in the potency of these combinations to decrease response rate versus break point, further demonstrating that decreases in break point occur independent of decreases in response rate. Finally, although isobolographic and dose-addition analyses revealed that two SR/mCPP combinations produced a syner-
gistic attenuation of PR responding for Ensure, only the 1:1 SR/mCPP combination (based on relative potency to decrease break point) produced statistically significant synergistic effects on both break point and rate. Even at this ratio, SR/mCPP was 3.2-fold more potent at decreasing break point than response rate. Taken together, these data strongly suggest that the ability of SR and mCPP alone or in combination to decrease response rate is neither necessary nor sufficient to decrease break point for a palatable food; the synergistic effects of SR/mCPP combinations on progressive ratio responding therefore probably reflect a unique interactive effect on motivation for palatable food versus gross behavior such as locomotor activity in this assay. A further assessment of SR/mCPP combination effects on spontaneous locomotor activity, Rotorod performance, or righting reflex might reveal whether the combinations that decreased break point in the present study also produce gross behavioral impairments in assays designed to exclusively measure motor performance.

Although the present data demonstrate that these two receptor systems interact to produce synergistic, quantifiable changes in palatable food self-administration, they do not specifically address the mechanism of action underlying these effects. Indeed, the site of action of SR141716 or mCPP alone in decreasing motivation for palatable food is not definitive because CB1 and 5-HT receptors in the hypothalamus, mesolimbic dopamine pathway, and in the periphery modulate feeding behavior. However, because the observed synergistic interaction of SR/mCPP coadministration involved a decrease in motivation as measured by operant responding for a palatable food reinforcer, this synergy probably involves modulation of activity within the mesolimbic dopamine system, which is generally accepted as the brain’s “reward circuit” integral to reinforced behavior. In contrast, Rowland et al. (2001) observed only additive decreases in ingestive behavior after SR141716/dexfenfluramine administration in rats using a free-feeding paradigm that may

![Fig. 3. Synergistic and additive effects of SR/mCPP combinations on break point. Filled symbols, predicted additive ED25 values (± S.E.M.) to decrease break point; open symbols, experimentally determined ED25 values (± S.E.M.) to decrease break point. Abscissas, ED25 value for SR141716 in milligrams per kilogram. Ordinates, ED25 value for mCPP in milligrams per kilogram. *, observed ED25 is statistically significantly different from predicted additive ED25.](image)

**TABLE 1**

<table>
<thead>
<tr>
<th>SR/mCPP Ratio</th>
<th>ED25 Predicted Additive (Zmix) ± S.E.M.</th>
<th>ED25 Observed (Zmix) ± S.E.M.</th>
<th>t′</th>
<th>t</th>
<th>Significant?</th>
<th>ED25 Observed, Actual Dose Pairs, SR ± S.E.M.;mCPP ± S.E.M.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1:1</td>
<td>1.24 ± 0.260</td>
<td>0.353 ± 0.068</td>
<td>3.28</td>
<td>2.29</td>
<td>Yes</td>
<td>0.311 ± 0.059;0.042 ± 0.009</td>
</tr>
<tr>
<td>2:1</td>
<td>1.64 ± 0.413</td>
<td>0.321 ± 0.120</td>
<td>3.06</td>
<td>2.29</td>
<td>Yes</td>
<td>0.302 ± 0.113;0.019 ± 0.007</td>
</tr>
<tr>
<td>3:1</td>
<td>1.79 ± 0.460</td>
<td>1.17 ± 0.320</td>
<td>1.11</td>
<td>2.36</td>
<td>No</td>
<td>1.12 ± 0.307;0.050 ± 0.013</td>
</tr>
<tr>
<td>1:2</td>
<td>0.965 ± 0.222</td>
<td>0.507 ± 0.025</td>
<td>2.06</td>
<td>2.20</td>
<td>No</td>
<td>0.401 ± 0.020;0.106 ± 0.005</td>
</tr>
<tr>
<td>1:3</td>
<td>0.798 ± 0.182</td>
<td>0.895 ± 0.345</td>
<td>−0.25</td>
<td>2.36</td>
<td>No</td>
<td>0.644 ± 0.248;0.251 ± 0.097</td>
</tr>
</tbody>
</table>
predominantly involve brain regions associated with hunger and satiety signals, such as the hypothalamus. Mounting evidence suggests that cannabinoid/serotonin neurochemical interactions are region-specific; that is, pharmacological manipulation of CB1 receptors can modulate serotonin efflux selectively within the mesocorticolimbic system (Tzavara et al., 2003) versus within the hypothalamus (Tzavara et al., 2001; Moranta et al., 2004). Finally, in addition to the hypothesis that the observed synergy depends upon unique neurochemical interactions, it is also possible that the coadministration of SR141716 and mCPP alters the pharmacokinetic profile of one or both agents. SR141716 is metabolized primarily by the CYP3A pathway, and concomitant administration of CYP3A inhibitors has been shown to increase plasma levels of SR (European Medicines Agency, European Public Assessment Report on Acomplia, http://www.emea.europa.eu/humandocs/PDFs/EPAR/acompilia/H-666-P1-en.pdf). mCPP is metabolized exclusively through the CYP2D6 pathway (Rotzinger et al., 1998) and has no known effect as a CYP3A inhibitor, making it unlikely that the metabolism of mCPP or SR141716 was inhibited in a manner significant enough to alter the behavioral observations in the present study.

Further studies aimed at elucidating the mechanism underlying synergistic interactions between the cannabinoid and serotonin systems on food reinforcement can increase our understanding of how multiple neural systems interact in the regulation and dysregulation of food reward. As pre-

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**Fig. 4.** Synergistic and additive effects of SR/mCPP combinations on response rate. The combination ratio corresponding to both the relationship of SR to mCPP based on relative potency to decrease break point and the relationship of SR to mCPP based on relative potency to decrease response rate are included above each isobologram. Closed symbols, predicted additive ED$_{25}$ values ($\pm$S.E.M.) to decrease response rate; open symbols, experimentally determined ED$_{25}$ values ($\pm$S.E.M.) to decrease response rate. Abscissas, ED$_{25}$ value for SR141716 in milligrams per kilogram. Ordinates, ED$_{25}$ value for mCPP in milligrams per kilogram. *, observed ED$_{25}$ is statistically significantly different from predicted additive ED$_{25}$.

**TABLE 2**

| SR/mCPP Ratio Based on Break Point/Response Rate | ED$_{25}$ Predicted Additive ($Z_{add}$) $\pm$ S.E.M. | ED$_{25}$ Observed ($Z_{mix}$) $\pm$ S.E.M. | $t$' | T | Significant?
<table>
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<tbody>
<tr>
<td>1:1/2:8:1</td>
<td>4.542 $\pm$ 1.320</td>
<td>1.125 $\pm$ 0.444</td>
<td>2.455</td>
<td>2.293</td>
<td>Yes</td>
</tr>
<tr>
<td>2:1/6:1</td>
<td>3.914 $\pm$ 1.348</td>
<td>1.038 $\pm$ 0.116</td>
<td>2.126</td>
<td>2.284</td>
<td>No</td>
</tr>
<tr>
<td>3:1/3:1</td>
<td>3.749 $\pm$ 1.389</td>
<td>1.649 $\pm$ 0.817</td>
<td>1.304</td>
<td>2.941</td>
<td>No</td>
</tr>
<tr>
<td>1:2/1.5:1</td>
<td>5.071 $\pm$ 1.455</td>
<td>1.589 $\pm$ 0.328</td>
<td>2.330</td>
<td>2.278</td>
<td>Yes</td>
</tr>
<tr>
<td>1:3/1:1</td>
<td>4.933 $\pm$ 1.407</td>
<td>1.856 $\pm$ 1.355</td>
<td>1.575</td>
<td>2.410</td>
<td>No</td>
</tr>
</tbody>
</table>
viously mentioned, the future of antiobesity treatment may lay in drug combination pharmacotherapies because currently available monotherapies for obesity are limited in number and effectiveness, producing an average weight loss in the range of 10% (5% better than placebo) (Bray and Greenway, 2007). Synergistic interactions between drugs from different classes that share overt behavioral effects (in this case, decreases in motivation for palatable food) not only suggests improved clinical effectiveness of combination therapy but may also allow for lower doses of each compound to be given, thus decreasing the potential for adverse effects. In the present study, quantifying cannabinoid-serotonin interactions across a range of relative proportions and two experimental endpoints revealed that 2:1 SR/mCPP was 5.8-fold more potent at decreasing break point and did so in a significantly synergistic manner compared with its effects on response rate. Regarding the clinical implications of using CB1 antagonists and 5-HT2C agonists in combination for future pharmacotherapies, it would be necessary to assess whether synergistic combinations of these compounds also produce an equally potent and/or synergistic production of anxiety and/or depressive symptoms, given that representative CB1 antagonist SR and 5-HT2C agonist mCPP have reportedly produced psychiatric side effects that have lessened their clinical utility (Cowen et al., 1995; Traynor, 2007). Likewise, the combined effects of other compounds within these two drug classes can be explored for synergistic interactions; for example, more recently, developed 5-HT2C agonists such as WAY 163909 show anorectic effects in rats while lacking the anxiogenic profile of mCPP and may even possess antidepressant-like activity (Dunlop et al., 2006).

The strengths of the present study are that it provides a quantitative assessment of the following: 1) the relative potency of five distinct SR/mCPP combinations; 2) which specific combinations act synergistically to decrease PR responding for palatable food; and 3) whether these combinations act synergistically and with the same potency to decrease refeeding for palatable food; and 4) whether these combinations act synergistically and with the same potency to decrease refeeding for palatable food; and 5) whether these combinations act synergistically and with the same potency to decrease refeeding for palatable food.

Acknowledgments

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