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Monoamine reuptake site occupancy of sibutramine: Relationship to antidepressant-like and thermogenic effects in rats

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ABSTRACT

Sibutramine was formerly marketed as an anti-obesity agent. The current study investigated the relationships between monoamine reuptake site occupancy for sibutramine and both its antidepressant-like efficacy and thermogenic effects. Sibutramine's effects on locomotor activity (LMA) and food intake were also evaluated. Sibutramine occupied monoamine reuptake binding sites with the rank order of potency of NET > SERT > DAT; at 10 mg/kg, po, occupancy was 95% NET, 81% SERT and 73% DAT. Sibutramine produced antidepressant-like behavior in the forced swim test; at the lowest effective dose (3 mg/kg, po) occupancy was 61%, 90% and 23% at SERT, NET and DAT sites, respectively. Sibutramine also increased body core temperature in a dose- and time-dependent manner; at the lowest effective dose (30 mg/kg) SERT, NET and DAT occupancies were respectively 78%, 86% and 59%. A significant decrease in food consumption was observed at 3 and 10 mg/kg, po. LMA was increased at ≥ 10 mg/kg, sc. The relationship between efficacy in the FST and occupancy was also determined for citalopram, fluoxetine and reboxetine. Similarly, the relationship between thermogenesis and target occupancy for several single or double/triple reuptake inhibitors was measured and showed that > 40–50% DAT binding was required for thermogenesis. Thermogenesis was blocked by the D_1 antagonist SCH39166 (3 mg/kg, sc). Our findings indicate that the antidepressant-like effect of sibutramine may result from additive or synergistic actions on the three reuptake binding targets. At higher doses, sibutramine produces thermogenesis; DAT inhibition and activation of dopamine D_1 receptors are required for this effect.

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1. Introduction

Sibutramine (Meridia) was initially developed as an antidepressant, but the unexpected observation of consistent weight loss in treated patients during the depression trials led to its subsequent development for the treatment of obesity (Kelly et al., 1995). Cardiovascular concerns, however, led to its discontinuation in many countries. Preclinical studies suggest that sibutramine produces weight loss by decreasing food intake, due to enhancement of satiety and suppression of hunger, and by increasing energy expenditure (Finer, 2002; Luque and Rey, 2002).

Sibutramine is a selective and relatively weak monoamine reuptake inhibitor. However, systemically administered sibutramine undergoes rapid metabolism to form two major metabolites, desmethylsibutramine and didesmethylsibutramine. Both metabolites exhibit substantially improved inhibitory potencies against norepinephrine (NE), serotonin (5-HT) and dopamine uptake (Glick et al., 2000; Heal et al., 1998; Nisoli and Carruba, 2000). The IC_{50} values for (R)-desmethysibutramine and (R)-didesmethysibutramine to inhibit NE, 5-HT and dopamine uptake function were, respectively, 4, 44 and 1 nM, and 13, 140 and 8.9 nM; the corresponding IC_{50} values for sibutramine were 0.35, 2.8 and 1.2 μM (Glick et al., 2000). Additionally, the active metabolites exhibit superior pharmacokinetic properties (i.e., higher and longer plasma exposure than sibutramine; Abolfathi et al., 2004). Consequently, the actions of the active metabolites are thought to largely underpin sibutramine's in vivo effects. Sibutramine's primary mechanism of action is commonly attributed to the inhibition of 5-HT and NE reuptake in central synapses (Luscombe et al., 1990; Glick et al., 2000), but microdialysis studies have demonstrated that systemically administered sibutramine also increases the concentration of dopamine, in addition to NE and 5-HT, in the brain (Invernizzi and Garattini, 2004; Rowley et al., 2000; Balcioglu and Wurtman, 2000). The effects on the NE and 5-HT systems are thought to underlie sibutramine's anorexic effect as blockade of adrenergic and serotonergic functions abolished this

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activity (Jackson et al., 1997; Finer, 2002; Liu et al., 2002b). Sibutramine's effects on the dopamine system may mediate its elevation of energy consumption. Specifically, the increased oxygen consumption and locomotor activity produced by sibutramine can be blocked by a dopamine D_1 antagonist (Golozoubova et al., 2006). These data suggest that systemically administered sibutramine may inhibit all three monoamine reuptake sites at doses that produce efficacy.

Interestingly, there is little information on the degree of target engagement produced by sibutramine and its active metabolites at doses producing pharmacological effects. A recent clinical PET study demonstrated that sibutramine occupied SERT sites in humans (Talbot et al., 2010). In rats, it has been shown that at doses that inhibited food intake sibutramine occupied a high level of NET sites with a very low SERT and nominal DAT occupancy (Thomas et al., 2009). Based on the earlier microdialysis and dopamine receptor blocking studies cited above, we anticipated that sibutramine administration would result in occupancy of DAT, as well as SERT and NET, at doses that produced antidepressant-like effects and thermogenesis. Thus, by using a sensitive ex vivo binding assay developed in our laboratory (Lengyel et al., 2008), we investigated the monoamine reuptake site occupancy of sibutramine in the rat brain, and determined the relationship of the target occupancy with the antidepressant-like and thermogenic effects.

2. Materials and methods

2.1. Drugs

Citalopram, desipramine, duloxetine, methylphenidate, reboxetine and sibutramine were purchased from Sigma-Aldrich (St. Louis, MO). Indatraline, GBR-12935 and SCH39166 were purchased from Tocris Bioscience (Ellisville, MO). [^{3}H]citalopram, [^{3}H]nisoxetine and [^{125}I]RTI-55 were purchased from Perkin Elmer Life Sciences (Boston, MA). DOV 21,947 was synthesized by the Chemical Synthesis Group at Bristol-Myers Squibb. All agents were freshly prepared in saline for subcutaneous (sc) administration or as a suspension in 0.25% methylcellulose in deionized water for oral administration (po), and were dosed in a volume of 2 ml/kg body weight. Doses were calculated and expressed in terms of free-base weight.

2.2. Animals

Male Sprague-Dawley (180–300 g) and Fischer rats (150–300 g) were purchased from Charles River Laboratories (Wilmington, MA). Upon arrival, they were housed singly or in pairs in polystyrene cages on Alpha-Dri bedding (Shepherd Specialty Papers, Kalamazoo, MI) at 22 °C and 50% humidity. They were maintained on a 12 h/12 h light/dark schedule with lights on at 0600 h, except for the overnight feeding study where lights were on at 0400 h. Rats received ad libitum water and food (rodent diet; PMI Nutrition International, Brentwood, MO). All experimental procedures were performed according to the protocols approved by the Animal Care and Use Committee of the Bristol-Myers Squibb Company and the published guidelines in the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

2.3. Animal dosing, brain tissue collection and radioligand binding for ex vivo occupancy assessment

In the studies where only reuptake occupancy was evaluated, rats were dosed with sibutramine at 0.3, 1, 3, 10 and 30 mg/kg, sc, in saline ($n=4$ per group). Sixty min post-dose, the rats were killed by decapitation, and after removal of the cerebellum and brainstem, the remaining brain tissues were rapidly frozen in chilled isopentane and stored at −80 °C until needed. In the thermogenesis and forced swim studies (see below for details) the rats were killed upon study completion and the brain tissues were collected as described above.

The method for assessing monoamine reuptake binding site occupancy has been described in detail in a previous publication (Lengyel et al., 2008). Briefly, on the day of occupancy assessment, the brain tissues were thawed and homogenized in 7–10 volumes of an assay buffer using a polytron homogenizer (Kinematica Inc., Newark, NJ). The assay solution contained 50 mM TRIS, 120 mM NaCl and 5 mM KCl (pH 7.4). Protein content was measured for each brain using a Coomassie protein assay kit (Pierce Chemical, Rockford, IL). In a 96-well plate, 100 μg of tissue (0.4 mg/ml) was incubated at 4 °C for 10–20 min with radioligand as follows: 2 nM [^{3}H]citalopram for SERT, 2 nM [^{3}H]nisoxetine for NET or 5 nM [^{125}I]RTI-55 (including 0.5 μM citalopram to block the SERT component of [^{125}I]RTI-55 binding) for DAT. The incubation solution for SERT and NET binding contained 50 mM Tris, 120 mM NaCl and 5 mM KCl, and that for DAT binding was 30 mM sodium phosphate buffer. The non-specific binding for SERT, NET and DAT sites was defined by inclusion of citalopram, reboxetine or GBR-12935, respectively (all at 10 μM). At the end of the incubation period, the reactions were stopped by filtration through FPXLR-196 filters (Brandel, Gaithersburg, MD) that had been soaked in 0.5–1.0% polyethyleneimine for 1 h at 4 °C. The filters were washed twice with ice-cold assay solution, and the radioactivity was measured using a Wallac Microbeta liquid scintillation counter (Perkin Elmer Life Sciences, Boston, MA).

2.4. Measurement of rectal temperature

Rats were acclimated for 3 days before they were tested for the thermogenic effects of sibutramine dosed at 10, 30, 100 mg/kg, sc or other agents. A temperature probe (YSI 401 micro temperature probe, King-Med, China) was inserted into the rectum of each rat and the temperature (representing core body temperature) was recorded using a VWR 61220-670 temperature reader (VWR International, Bridgeport, NJ) at 0, 1, 2, 3 and 4 h post-dose. Immediately after the last temperature measurement, the rats were killed and brain tissues were collected as described above for assessment of ex vivo occupancy.

2.5. Forced swim test

A modified rat forced swim test (FST; Detke et al., 1995) was used to determine the antidepressant-like effect of sibutramine, citalopram, fluoxetine and reboxetine. Briefly, on day one, rats were placed individually in Pyrex cylinders (46 cm tall × 21 cm in diameter) filled with water to 30 cm depth at 25 °C. The rats were removed 5 min later, dried and placed in their home cage. On day two, 24 h after the first exposure, the rats were again placed in the swim apparatus for 5 min and behaviors were monitored from above by video camera for subsequent analysis. Animals were orally dosed with sibutramine (1, 3 or 10 mg/kg), citalopram (1, 3 or 10 mg/kg), fluoxetine (10 or 25 mg/kg) or reboxetine (3, 10 or 30 mg/kg) three times at 23.5, 5 and 1 h prior to the test session (3 dose paradigm). A separate group of animals was given a single oral dose of 25 mg/kg fluoxetine at 60 min prior to the test session. Three predominant behaviors were recorded in each 5 s period of the 300 s test. Climbing behavior was defined as upward movements of the forepaws along the edge of the swim chamber. Swimming behavior was identified as horizontal movements throughout the cylinder. Immobility was described when no additional activity was