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Pharmacology, Biochemistry and Behavior 80 (2005) 521–528

PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR

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Neurochemical, pharmacokinetic, and behavioral effects of the novel selective serotonin reuptake inhibitor BMS-505130

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Received 4 June 2004; received in revised form 15 January 2005; accepted 17 January 2005

Abstract

BMS-505130 is a potent and selective serotonin transport inhibitor; K_i for binding to the serotonin transporter=0.18 nM (K_i values for binding to the norepinephrine and dopamine transporters=4.6 and 2.1 μM, respectively). In platelet serotonin uptake studies BMS-505130 (5 mg/kg, p.o.) produced a robust inhibition of serotonin uptake. In microdialysis studies oral dosing with BMS-505130 produced a dose-dependent increase in cortical serotonin levels that reached a maximal effect of 200% above baseline at a dose of 1 mg/kg, p.o.; the peak serotonin response was transient in nature. Following oral administration, peak plasma concentrations of BMS-505130 reached Tmax at 1.6±0.7 h and then declined to concentrations <10% of Cmax within the following 6 h; plasma half-life following i.v. dosing was 0.46±0.02 h. Parallel microdialysis and pharmacokinetic studies revealed that changes in serotonin levels in the cortex mirrored changes in the brain concentration of BMS-505130. In a behavioral assay known to be sensitive to selective serotonin reuptake inhibitors (SSRIs), mouse tail suspension, BMS-505130 produced a robust response after either oral or intraperitoneal dosing. BMS-505130 exhibits a pharmacological, neurochemical and behavioral profile consistent with a potent SSRI. Moreover, BMS-505130's short half-life may be advantageous for the treatment of premature ejaculation where an acute effect to delay ejaculation followed by a relatively rapid fall in SSRI plasma concentrations might be desirable.

Keywords: BMS-505130; Microdialysis; Selective serotonin reuptake inhibitor (SSRI); Serotonin transporter (SERT); Tail suspension test

1. Introduction

Premature ejaculation (PE) is a common problem that affects ~30% of men (Laumann et al., 1999). This disorder was originally viewed as either a neurosis or a learned behavior and consequently was treated using classical psychoanalysis and behavioral therapy (Waldinger, 2002). Modern treatment of PE involves behavioral or pharmacological treatment. Behavioral therapies (e.g. squeeze, stop–start) have been reported to produce mixed results and tend to offer only a short-term benefit. Pharmacological treatments for PE have included the use of local anesthetics, tricyclic antidepressants, and selective serotonin reuptake inhibitors (SSRIs: Waldinger, 2002). The tricyclic antidepressant chlomipramine has been shown to delay ejaculation in humans (Haensel et al., 1996). However, its utility is limited by adverse reactions such as drowsiness, dry mouth, blurred vision, and other anticholinergic side effects.

More recently, SSRIs such as paroxetine, fluoxetine, and sertraline, have also been shown to be effective for the treatment of PE (Waldinger et al., 1998a; McMahon and Samalim, 1999; Manasia et al., 2003). Citalopram was effective in some studies (Atmaca et al., 2002) but marginal

Abbreviations: nPG, nucleus paragigantocellularis; PE, premature ejaculation; SSRI, selective serotonin reuptake inhibitor; Cmax, maximum plasma concentration.

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doi:10.1016/j.pbb.2005.01.007

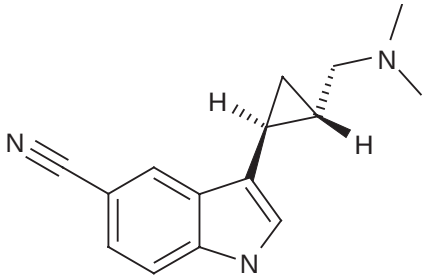

Fig. 1. Chemical structure of BMS-505130.

in others (Waldinger et al., 1998b). Typically, SSRIs must be administered for a minimum of four weeks to achieve maximal antidepressant effects. Interestingly, however, several small clinical trials have now shown that SSRIs can be effective when used on an as needed basis. For example, paroxetine effectively extends latency to ejaculation when taken 3 to 4 h prior to intercourse, a dosing regimen that also produced no adverse effects (McMahon and Touma, 1999b). An initial period of daily paroxetine followed by "as needed" dosing produced further improvement (McMahon and Touma, 1999a,b). The prototypical SSRI fluoxetine may also be effective in an intermittent dosing regimen; Manasia et al. found that fluoxetine, given either daily (20 mg) or once a week (90 mg) had equivalent efficacy in delaying ejaculation compared with placebo (Manasia et al., 2003). Lastly, indirect support for an acute action of the SSRIs on sexual function may be drawn from the observation that a "drug holiday" (no dose Friday and Saturday morning, next dose Sunday noon) from the chronic use of SSRIs (sertraline and paroxetine, but not fluoxetine) resulted in improved sexual functioning and no decrease in Hamilton depression scores (Rothschild, 1995).

The current paper describes the pharmacology, neurochemistry, pharmacokinetics, and behavioral effects of a new SSRI, BMS-505130 (Fig. 1). BMS-505130 is a potent and selective serotonin transport inhibitor that exhibits a short half-life that may be advantageous for disorders such as PE that may respond to an acute and intermittent exposure to an SSRI.

2. Methods

2.1. Binding studies

2.1.1. Serotonin transporter binding assay

Membrane was prepared for binding using the human serotonin transporter expressed in HEK293 cells. Cells were collected and ruptured using a dounce homogenizer. Homogenates were centrifuged at 32,000×*g* for 10 min and the pellet was resuspended in assay buffer, frozen in liquid nitrogen, and kept at −80 °C until the day of the assay.

The assay was carried out in 96-deep-well plates using a total of 10 μg protein per well. The assay buffer was 50 mM TRIS, pH 7.4, containing 120 mM NaCl and 5 mM KCl. Membrane was incubated at 25 °C for 60 min with test compound and 2 nM [^{3}H]-citalopram. Non-specific binding was defined with 10 μM fluoxetine. The reaction was terminated by the addition of 1 ml of ice-cold 50 mM TRIS buffer and rapid filtration through a Brandel Cell Harvester using Whatman GF/B filters presoaked in 1.0% PEI. The filter pads were counted in a LKB Trilux liquid scintillation counter. IC_{50} values were determined using non-linear regression by Microsoft Excel-fit. IC_{50} values were corrected to K_i values using the method of Cheng and Prusoff (1973).

2.1.2. Norepinephrine transporter binding assay

Membrane prepared from MDCK cells expressing the human norepinephrine transporter was purchased from Perkin Elmer Life Sciences. The assay buffer was 50 mM TRIS (pH 7.4 at 25 °C) containing 120 mM NaCl and 5 mM KCl. Membrane (8 μg/well) was incubated on ice for 60 min with test compound and 2.7 nM [^{3}H]-nisoxetine in a 96-deep-well plate. Non-specific binding was defined with 10 μM desipramine. The reaction was terminated by the addition of 1 ml of ice-cold 50 mM TRIS buffer and rapid filtration through a Brandel Cell Harvester using Whatman GF/B filters presoaked in 0.5% polyethylenimine. The filter pads were counted in a LKB Trilux liquid scintillation counter. IC_{50} values were determined using non-linear regression by Microsoft Excel-fit. IC_{50} values were corrected to K_i values using the method of Cheng and Prusoff (1973).

2.1.3. Dopamine transporter binding assay

Membrane prepared from CHO cells expressing the human dopamine transporter was purchased from Perkin Elmer Life Sciences. Membrane (10 μg/well) was incubated 2 h at 4 °C with test compound and 5 nM WIN 35,428, [*N*-Methyl-^{3}H]-in assay buffer consisting of 30 mM sodium phosphate buffer (pH 7.9 at 4 °C). The reaction, carried out in 96-deep-well plates, was terminated by the addition of 1 ml ice-cold assay buffer and rapid filtration through a Brandel Cell Harvester using Whatman GF/B filters presoaked in 0.5% polyethylenimine followed by three additional 1 ml filter washes. Non-specific binding was defined with 5 μM GBR-12935. The filter pads were counted in a LKB Trilux liquid scintillation counter. IC_{50} values were determined using non-linear regression by Microsoft Excel-fit. IC_{50} values were corrected to K_i values using the method of Cheng and Prusoff (1973).

2.1.4. 5-HT_{1A} Receptor binding assay

Membranes were prepared from HEK293 cells expressing the human 5-HT_{1A} receptor. Cells were collected and ruptured using a dounce homogenizer. Homogenates were centrifuged at 32,000×*g* for 10 min and the pellet was resuspended in assay buffer, frozen in liquid nitrogen, and kept at −80 °C until the day of the assay.

The assay was carried out in 96-deep-well plates using a total of 30 μg protein per well. The assay buffer was 50 mM HEPES containing 2.5 mM $MgCl_2$ and 2 mM EGTA (pH

7.4). The membrane preparation was incubated at 25 °C for 60 min with test compound and 1 nM [^{3}H]-8-OH-DPAT. Serotonin (10 μM) served as blocking agent to determine non-specific binding. The reaction was terminated by the addition of 1 ml of ice-cold 50 mM HEPES buffer and rapid filtration through a Brandel Cell Harvester using Whatman GF/B filters. The filter pads were counted in a LKB Trilux liquid scintillation counter. IC_{50} values were determined using non-linear regression by Microsoft Excel-fit.

2.2. Platelet studies

Male Sprague–Dawley rats (Harlan or Hilltop Lab Animals) weighing 280–330 g were used for all platelet and microdialysis experiments. For all of the in vivo studies, animals were group housed in an AALAC accredited facility and had ad libitum access to food and water at all times, except for overnight fasting the night prior to dosing. Animals were fasted to reduce variability in drug absorption due to stomach contents. All methods were approved by the Bristol-Myers Squibb Animal Care and Use Committee, and adhere to the guidelines indicated by the Guide for the Care and Use of Experimental Animals published by the National Institutes of Health (Pub. 85–23, 1985).

The method used for the rat platelet serotonin uptake studies was a modification of the method developed by Meltzer et al. for use in humans (Arora and Meltzer, 1981). The experiment included four groups of rats: control (n=8), or BMS-505130 (5 mg/kg p.o.) dosed 30 min (n=5), 60 min (n=5), or 120 min (n=6) prior to euthanitization. Following euthanitization by carbon dioxide inhalation, a cardiac puncture was performed and 1 ml of whole blood was collected into 1.5 ml Eppendorf tubes containing EDTA as an anticoagulant. The blood samples were centrifuged at 300×g for 10 min, and 300 μl of platelet rich plasma was collected for each sample. All plasma samples were pre-incubated for 2 min at 37 °C, then experimental samples were incubated for 1 min at 37 °C in [^{3}H]5-HT/5-HT (0.6 μM). Control samples (for nonspecific uptake) were incubated for 1 min at 0 °C in [^{3}H]5-HT/5-HT (0.6 μM). The reaction was terminated with 2 washes of 1 ml of saline at 0 °C plus centrifugation at 1900 rpm for 15 min. Two 60 min incubations at 50 °C were then performed, each following addition of Soluene 350 (0.5 ml) to liberate the [^{3}H]5-HT that had undergone uptake by the cells. Finally, 15 ml of scintillation cocktail was added to each sample, and activity was counted using a LKB trilux liquid scintillation counter. Specific activity was defined as the experimental minus the control samples. Specific activity was then calculated as percentage of pretreatment baseline, and results were analyzed with ANOVA followed by Dunnett's test.

2.3. Microdialysis studies

The surgery and microdialysis technique were adaptations of well established methods (Carboni et al., 1989; Taber et al., 2000). Male Sprague–Dawley rats were anesthetized with isoflurane by inhalation (2–5% by calibrated vaporizer) and placed in a stereotaxic apparatus. A transverse incision was made across the skull, the lateral surfaces of the skull adjacent to bregma were cleared of overlying tissue, and trephine holes were drilled on both sides (diameter=2.3 mm). Transverse microdialysis probes (Hospal, AN69) with an active dialysis length of 9.5 mm were inserted into the frontal cortex at the coordinates AP: 0.0, DV: −2.2 relative to bregma, according to the atlas of Paxinos and Watson (1986). The ends of the dialysis probes were capped with 22 gauge cannulae and attached to the skull with three screws and dental cement. The incision was sutured shut, and the animals were transferred to testing chambers for recovery.

Approximately 40–48 h after the surgery, artificial cerebrospinal fluid containing NaCl (147 mM), KCl (3 mM), $CaCl_2$ (1.2 mM), and $MgCl_2$ (1 mM) in water was perfused through the dialysis probe at a rate of 2 μl/min. Dialysate samples were analyzed with on-line high performance liquid chromatography (HPLC) coupled to electrochemical detection. The outlet from the probe was attached to 50 μl loop of an on-line injector (Valco) and samples were automatically injected onto an HPLC system once every 20 min. For separation with the HPLC system, mobile phase (50 mM sodium acetate, 4 mM octane sulfonic acid, 0.01 mM ethylene diamine tetraacetic acid, 1 ml/l triethylamine, and 17% MeOH, pH 4.2 adjusted with glacial acetic acid) were delivered by HPLC pump (model ESA 580, ESA, Chelmsford MA) at 0.4 ml/min. Separation was achieved with a reverse phase column (ESA MD-180, 3×150 mm, 3 μm particles). Serotonin was quantified with an electrochemical detector (Coulochem II, ESA) and a flow-through cell (model 5014B, ESA) using sequential reduction (−100 mV) and oxidation (+220 mV). Output from the detector was recorded with a computerized system (ESA 500 Datastation) programmed to integrate the serotonin peak recorded at the oxidizing electrode.

After the establishment of a baseline, drug treatments were administered and serotonin concentrations were recorded for an additional 5 h. All drugs were administered orally in a volume of 1 ml/kg. For analysis, all data were represented as a percentage of baseline, and statistical analyses were performed with a repeated measures ANOVA using Huyn–Felt adjustments to account for time as a repeated measure.

2.4. Pharmacokinetic studies

Male Sprague–Dawley rats (300–350 g, Hilltop Lab Animals, Scottsdale, PA 15683) with cannulae implanted in the jugular vein by the vendor were used in the pharmacokinetic studies of BMS-505130. The compound was dosed in water. Blood samples (0.3 ml) were collected from the jugular vein in EDTA microtainer tubes (Becton Dickinson,

Franklin Lakes, NJ 07147) to obtain plasma. Plasma samples were stored at −20 °C until analysis.

For brain uptake studies, rats were fasted overnight and received an oral dose of 1 mg/kg. Animals (n=2 per time point) were sacrificed at 10, 30, 50, 110, 170, 230, 290, and 350 min after dosing, at which point brain and plasma samples were obtained. These time points were selected to be at the midpoint of the collection times for the microdialysis samples.

The brain was removed and samples were rinsed with normal saline, weighed, and placed on ice. The brain samples were then stored frozen (−20 °C) until homogenized in 4.0 ml of acetonitrile and the volume of the homogenate measured. The homogenates were centrifuged at 2500 rpm for 10 min, and a 1 ml aliquot of the supernatant was submitted for analysis. The study samples and standards in brain homogenate were extracted and analyzed by LC/MS/MS.

2.5. Mouse Tail Suspension

The Mouse Tail Suspension Test used is a variant of the one originally described by Steru et al. (1985). Male BalbC mice (Harlan Sprague–Dawley) weighing 20–25 g and housed in polycarbonate cages, 4 per cage, with free access to food and water were used in each experiment. All experiments were performed between 8 AM and noon, during the light phase (lights on 6 AM to 6 PM). Mice were randomly assigned to drug or vehicle groups (n=8–11 per group); drug or vehicle was administered by intraperitoneal injection or oral gavage (injection/gavage volume=10 ml/kg). Thirty or 60 min following treatment the mice were individually suspended by the tail to a strain gauge. The time spent immobile (s) during a 6 min assessment period was automatically recorded by an ENV-505TS system from MED Associates. Statistical analyses were performed utilizing an ANOVA followed by a post-hoc Dunnett's test (α=0.05).

2.6. Compounds

Fluoxetine HCl was synthesized at Bristol-Myers Squibb using standard methodology or purchased from RBI (tail suspension studies). Paroxetine HCl was purchased commercially in capsule form, and was isolated by chemists at Bristol-Myers Squibb using standard methods. BMS-505130 (maleate salt) was synthesized at Bristol-Myers Squibb. All compounds were dosed as the salt forms using distilled water as the vehicle.

3. Results

3.1. Binding studies

BMS-505130 was found to bind potently to the human serotonin transporter (K_i=0.18 nM; Fig. 2; Table 1). In contrast, BMS-505130 bound to the norepinephrine and dopamine transporters with much lower affinity (K_i=4.6 and 2.1 μM, respectively; Fig. 2; Table 1). BMS-505130 also bound to human 5-HT_{1A} receptors with low affinity (K_i=410 nM). Comparative binding studies revealed that BMS-505130 exhibited similar potency to fluoxetine and paroxetine at the serotonin transporter, but was more selective for the serotonin transporter relative to the norepinephrine and dopamine transporters (Table 1).

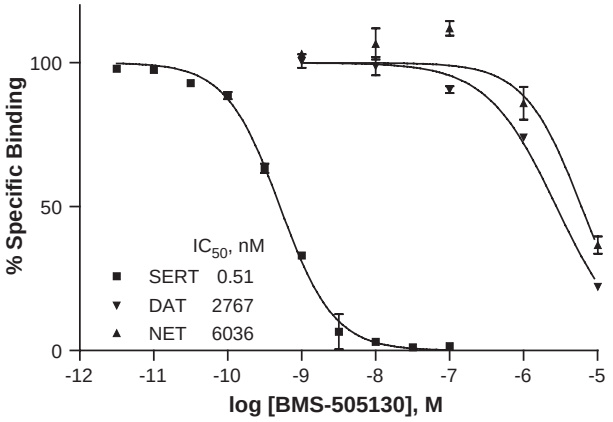

Fig. 2. Inhibition by BMS-505130 of: [^{3}H]-citalopram binding to cloned human serotonin transporters expressed in HEK-293 cells (squares), [^{3}H]-WIN-35,428 binding to cloned human dopamine transporters expressed in CHO cells (downward triangles), and [^{3}H]-nisoxetine binding to cloned human norepinephrine transporters expressed in MDCK cells (upward triangles). Values represent means±S.E.M. of 2–12 determinations.

3.2. Platelet serotonin uptake

BMS-505130 produced a statistically significant inhibition of serotonin uptake into platelets isolated from rats dosed orally with BMS-505130 (p<0.05 by ANOVA); 88, 97, and 94% inhibition of serotonin uptake was observed at 0.5, 1, or 2 h post-dosing, respectively (Fig. 3). Thus, BMS-505130 produced a rapid and robust effect on platelet serotonin transport following oral dosing.

3.3. Microdialysis studies

BMS-505130 produced robust, dose-dependent increases in extracellular serotonin in the frontal cortex (p< 0.001 by repeated measures ANOVA; Fig. 4). Doses of 1.0 and 3.0 mg/kg p.o. produced effects that were significantly greater than vehicle (p<0.05, two-way repeated measures ANOVA), whereas the effects of 0.3 and 0.1 mg/kg were not statistically significant. Comparative studies revealed that the maximum effect of paroxetine observed during the 4 h period following oral administration was achieved with a dose of 5 mg/kg (see Fig. 5). The amplitude of the serotonin response reached ~150% above baseline, consistent with many previous studies that have established that SSRIs produce a maximal response of 100–200% above baseline (for example: Beyer et al., 2002). Similar studies showed

Table 1
K_i values for BMS-505130, paroxetine, and fluoxetine

	*h*SERT K_i, nM (*n*)	*h*NET K_i, nM (*n*)	*h*DAT K_i, nM (*n*)
BMS-505130	0.18±0.02 (12)	4588 (2)	2123 (2)
Paroxetine	0.037±0.003 (18)	94 (2)	382 (2)
Fluoxetine	0.72±0.05 (24)	444 (2)	1922 (2)

that fluoxetine produced a maximum serotonin response at a dose of 10 mg/kg (data not shown). As can be seen in Fig. 6, the maximum serotonin responses produced by BMS-505130 were similar to that of paroxetine and fluoxetine. The maximum serotonin response for BMS-505130 was achieved 60 min after drug administration whereas the maximal effects for paroxetine and fluoxetine were achieved much later, 160 min or more after dosing. Moreover, the peak serotonin response to BMS-505130 was transient in nature (Figs. 4 and 6), consistent with its pharmacokinetic properties (see below).

3.4. Pharmacokinetic studies

Following oral administration in the rat, plasma concentrations of BMS-505130 reached peak plasma levels by 1.6±0.7 h and then declined to concentrations <10% of Cmax within the following 6 h (n=3; Fig. 7). Following i.v. dosing plasma levels declined with a half-life of 0.46±0.02 h (n=3; Fig. 7); oral bioavailability was 56%.

To compare the time course of changes in brain concentrations of serotonin with the time course of brain concentrations of BMS-505130, an additional microdialysis study was performed in parallel with a pharmacokinetic study that measured brain and plasma levels of BMS-505130. A dose of 1 mg/kg p.o. was chosen as the minimal dose that produced maximal effects in the microdialysis dose response (Fig 8). The rise and subsequent fall in the concentration of BMS-505130 in the brain paralleled the time-course of the BMS-505130 induced changes in serotonin observed in the accompanying microdialysis experiments. The peak brain concentration of BMS-505130 was achieved 30 min after dosing whereas the peak

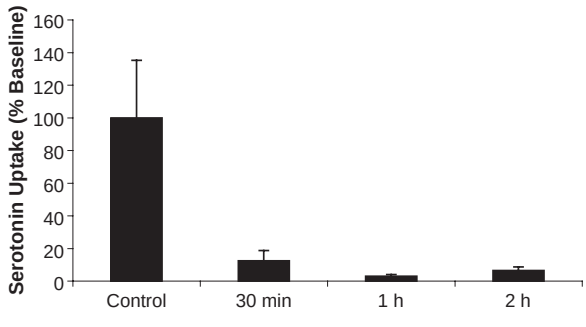

Fig. 3. Inhibition of [^{3}H]-serotonin uptake into rat platelets taken from control animals or animals previously treated with BMS-505130 (5 mg/kg p.o.) at the indicated interval prior to tissue collection. Values represent the mean±S.E.M. for n=5–8 per group.

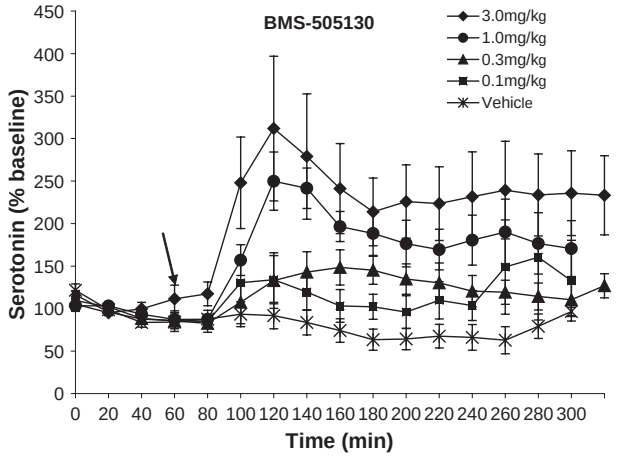

Fig. 4. Effects of BMS-505130 on extracellular serotonin levels in the frontal cortex of rats measured by microdialysis. Data points represent mean±S.E.M. of 4–6 rats/group, and the arrow indicates the time of compound administration. BMS-505130 was administered via oral gavage in a volume of 1 ml/kg to rats fasted overnight.

serotonin effect occurred at 60 min after dosing. Both responses decayed in parallel until reaching a level indistinguishable from baseline 6–7 h after dosing. Surprisingly, a second peak in brain serotonin concentrations was observed about 5 h after dosing. This effect corresponded with the administration of food to animals that had been fasted for 24 h. The response may not be a direct result of eating, however, as the food administration also caused the animals to locomote and in many cases to awaken from (apparent) sleep. It is possible that BMS-505130 interacted with feeding to produce a serotonin response as feeding in vehicle-treated animals did not produce a response.

3.5. Tail suspension

The mouse tail suspension test is a commonly used method to assess antidepressant efficacy. BMS-505130 produced a significant decrease in immobility time at doses

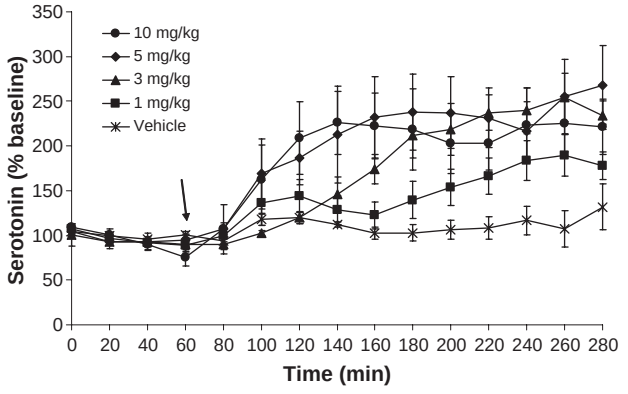

Fig. 5. Effects of paroxetine on extracellular serotonin levels in the frontal cortex of rats measured by microdialysis. Data points represent mean±S.E.M. of 4–6 rats/group, and the arrow indicates the time of compound administration. Paroxetine was administered via oral gavage in a volume of 1 ml/kg to rats fasted overnight.

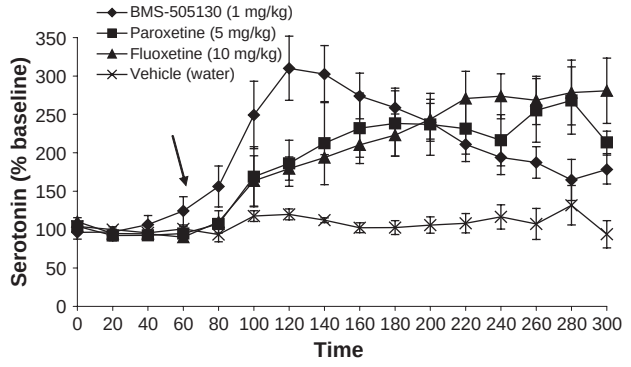

Fig. 6. Comparison of the effects of BMS-505130, fluoxetine, and paroxetine on extracellular serotonin levels in rat frontal cortex as measured by microdialysis. Data points represent mean±S.E.M. of 4–6 rats/group, and the arrow indicates the time of administration.

of 10 and 30 mg/kg i.p. or p.o. when dosed 60 min prior to testing (Fig. 9). Fluoxetine (i.p.) also produced a significant decrease in immobility time at doses ≥20 mg/kg (data not shown).

4. Discussion

BMS-505130 exhibits a pharmacological, neurochemical, and behavioral profile consistent with a potent SSRI. Its binding selectivity for the serotonin transporter relative to its binding potency at the norepinephrine and dopamine transporters is somewhat better than paroxetine and fluoxetine; it should be noted, however, that both paroxetine and fluoxetine are likely to act selectively at the serotonin transporter at therapeutic plasma concentrations. To confirm that the in vitro profile of BMS-505130 resulted in an appropriate in vivo response, rat platelet studies and microdialysis studies were performed. For the platelet studies BMS-505130 was administered orally, and platelets were collected at 30, 60, and 120 min after dosing from separate groups of rats. Ex vivo analysis of the platelet [^{3}H]-serotonin uptake demonstrated that the compound produced a robust inhibition (~90%) of serotonin uptake. The response was maximal at the earliest time point assessed (30 min), and remained at the maximal level for the duration of the 2 h experiment. This study confirmed that the binding of BMS-505130 seen in vitro corresponded to functional blockade of the serotonin transporter in vivo. To further characterize the effects of BMS-505130, microdialysis studies compared the efficacy and time course of the inhibition of serotonin uptake in vivo between BMS-505130 and reference agents. BMS-505130 produced a concentration-dependent increase in serotonin with a maximal response equal to that of fluoxetine and paroxetine. However, in contrast to fluoxetine and paroxetine (Figs. 5 and 6), the peak serotonin response to BMS-505130 was transient in nature as measured during this 4 h experiment (Figs. 4 and 6).

BMS-505130 has a relatively short plasma half-life (Fig. 7), especially when compared to other SSRIs; for example,

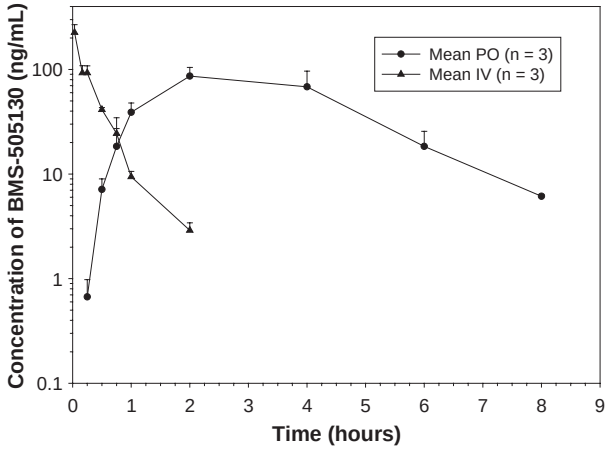

Fig. 7. BMS-505130 plasma concentrations following i.v. (1 mg/kg) and oral (5 mg/kg) dosing in rats (n=3).

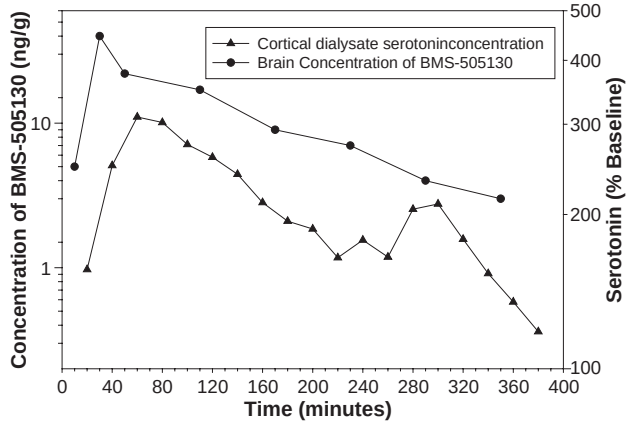

Fig. 8. Overlaid plots from parallel experiments showing brain concentrations of BMS-505130 (left axis) and cortical dialysate concentrations of serotonin (right axis) following oral administration of BMS-505130 (1 mg/kg p.o.).

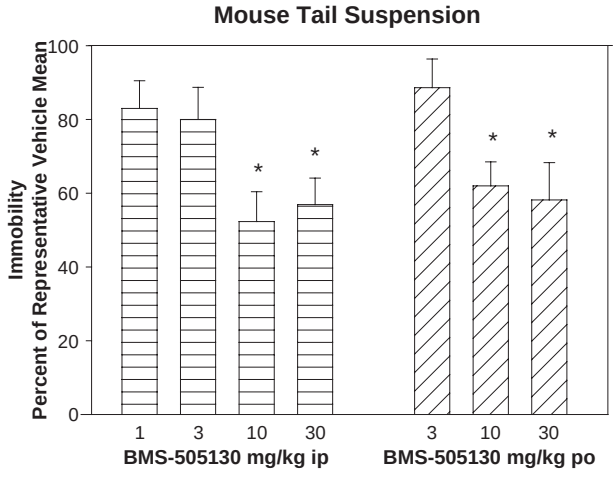

Fig. 9. Comparison of the effects of BMS-505130 i.p. and p.o. (60 min pretreatment) on immobility behavior in the Mouse Tail Suspension Test. (*p<0.05 vs. vehicle control).

paroxetine's half-life following oral dosing is ~21 h (in humans). To assess whether the relatively short duration of action of BMS-505130 was due to pharmacokinetic parameters or a pharmacological response such as desensitization, a study was performed to directly compare the kinetics of the changes in serotonin concentrations to the brain concentrations of BMS-505130. This study demonstrated that the time course of the rise and fall in extracellular serotonin closely paralleled the rise and fall in BMS-505130 concentration in the brain. The close temporal relationship between serotonin levels and the concentration of BMS-505130 in the brain indicates that the transient nature of the serotonin peak is most likely due simply to the pharmacokinetics of the compound rather than a negative feedback process such as that produced by the activation of 5-HT_{1A} and 5-HT_{1B} autoreceptors (Beyer et al., 2002).

The mouse tail suspension model, which is known to be sensitive to SSRIs, was used to establish whether the BMS-505130 induced changes in brain serotonin levels resulted in a behavioral outcome. BMS-505130 produced a significant decrease in immobility 60 min after i.p. or p.o. dosing, a response consistent with its neurochemical and pharmacokinetic properties. The decrease in immobility is thought to correlate to antidepressant efficacy.

The data described above establish that BMS-505130 is a potent functionally active SSRI. The short half-life of the compound may be advantageous for the treatment of PE, a disorder that is known to respond to SSRIs. Clinical studies demonstrate that treatment regimens that produce antidepressant responses, i.e. 4–6 weeks of dosing, are also useful for PE (Waldinger et al., 1998a; McMahon and Samalim, 1999; Manasia et al., 2003). Recent studies indicate that SSRIs can also produce beneficial effects on PE when dosed on short-term or PRN schedule. For example, paroxetine produced a clinically useful response when dosed as needed, 2 h prior to intercourse (McMahon and Touma, 1999a,b). Thus, SSRIs can act acutely to delay ejaculation; a property that would potentially allow the on-demand use of SSRIs for the treatment of PE. Given that the SSRIs as a class produce a number of side effects such as nausea and sleep disturbances, it may be advantageous to treat PE with a short half-life SSRI which would be expected to delay ejaculation acutely but then fall to low concentrations thereby reducing the long-term side effect burden.

Many studies have established that SSRIs produce a maximal response of 100–200% above baseline serotonin levels (Beyer et al., 2002). This ceiling effect is a result of activation of 5-HT_{1A} and 5-HT_{1B} autoreceptors, as evidenced by potentiation of serotonin responses by 5-HT_{1A} and 5-HT_{1B} antagonists (Beyer et al., 2002). The doses of BMS-505130, fluoxetine, and paroxetine required to produce a maximum increase in serotonin were determined. These studies allowed us to determine the *minimum* dose of each SSRI required to produce the maximum serotonin response for each compound. As can be seen in Fig. 6 the selected dose of BMS-505130 produced a considerably more rapid response than the selected doses of paroxetine or fluoxetine. Clearly, higher doses of either paroxetine or fluoxetine beyond those required for a maximum serotonin response would likely produce a more rapid increase in serotonin. The relatively rapid increase in serotonin produced by BMS-505130 at the minimum dose required to produce a maximum effect may also be advantageous as it might reduce the need to use a dose above that required for the maximum effect to obtain a fast rise in serotonin.

Many studies have employed rodent sexual behavior models to assess the effects of SSRIs on parameters of sexual function (Ahlenius and Larsson, 1999; Cantor et al., 1999; Waldinger et al., 2002). However, it is not clear that these models should be considered predictive of human clinical response. For example, the rodent models require chronic dosing whereas recent studies in patients indicate that on-demand treatment is effective in patients (as discussed above). Also, given the qualitative differences between rodent and human sexual behavior and the apparent differences in the physiology of the response (discussed below), attempts to draw parallels must be considered carefully.

Although the mechanism by which SSRIs mediate delay of ejaculation is not known, an intriguing possibility involves an increase in activity in the serotonergic cells within the nucleus paragigantocellularis (nPG). This nucleus contains serotonergic cells and is an origin of descending inhibitory influences on sexual function mediated by spinal reflexes (Marson and McKenna, 1990; Yells et al., 1994). Specifically, activity in this serotonergic pathway is thought to produce a tonic inhibition of ejaculation (Marson and McKenna, 1990; Yells et al., 1994). Furthermore, lesions to the nPG of the rat have been shown to partially inhibit the increased ejaculatory latency seen with fluoxetine in the sham animals (Yells et al., 1994). The nPG is also well situated to mediate serotonin/oxytocin interactions which may be critical for the regulation of sexual responses. Oxytocin administration facilitates and oxytocin receptor antagonists disrupt sexual responses in rats (Argiolas et al., 1987; Arletti et al., 1985). Importantly, oxytocin can also reverse the deficits in sexual performance in rats produced by an SSRI (Cantor et al., 1999). The oxytocin-mediated effect on sexual behavior most likely occurs via hypothalamic mechanisms, and as an area receiving afferents from the hypothalamus, the nPG could easily serve to mediate oxytocin/SSRI interactions (Waldinger, 2002).

The data discussed above address systems identified in rodent studies, but it is important to consider the degree to which these results correspond to higher species. In rodents, cFos immunohistochemistry studies have demonstrated that the medial preoptic area, bed nucleus of the stria terminalis, hypothalamic nuclei, and amygdalar nuclei are the foci of increases in neuronal activity during male ejaculation (Baum and Everitt, 1992; Heeb and Yahr, 1996). This finding is consistent with lesion studies in rats discussed

above. Importantly, however, a primate study found *decreases* or no effect on Fos expression in these areas, suggesting that the pattern of activity during ejaculation may be species specific (Michael et al., 1999). To address this question in humans, Holstege et al. have recently reported on the pattern of brain activation as measured with regional cerebral blood flow in male humans during ejaculation with PET imaging (Holstege et al., 2003). The highest level of activation encountered was in the meso-diencephalic transition zone, a region that contains the ventral tegmental area, among other nuclei. The ventral tegmental area is the origin of the mesolimbic dopamine system which is well established as a key nuclei mediating reward responses including the "rush" obtained from drugs of abuse. In contrast, no activation was observed in the areas predicted by the rodent studies such as the medial preoptic area, and bed nucleus of the stria terminalis (Holstege et al., 2003). These results may suggest that in humans, the SSRIs could serve to modulate ejaculation via effects on the midbrain dopamine system.

Acknowledgments

The authors gratefully acknowledge the following for providing excellent technical assistance: Billy Akinsanya, Melissa Cunningham, Gail Mattson, Amy Newton, Jennifer Pizzano, and John Torrente.

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