LP-211 is a brain penetrant selective agonist for the serotonin 5-HT$_7$ receptor

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Abstract

We have determined the pharmacological profile of the new serotonin 5-HT$_7$ receptor agonist *N*-(4-cyanophenylmethyl)-4-(2-diphenyl)-1-piperazinehexanamide (LP-211). Radioligand binding assays were performed on a panel of 5-HT receptor subtypes. The compound was also evaluated *in vivo* by examining its effect on body temperature regulation in mice lacking the 5-HT$_7$ receptor (5-HT$_7^{-/-}$) and their 5-HT$_7^{+/+}$ sibling controls. Disposition studies were performed in mice of both genotypes. It was found that LP-211 was brain penetrant and underwent metabolic degradation to 1-(2-diphenyl)piperazine (RA-7). *In vitro* binding assays revealed that RA-7 possessed higher 5-HT$_7$ receptor affinity than LP-211 and a better selectivity profile over a panel of 5-HT receptor subtypes. *In vivo* it was demonstrated that LP-211, and to a lesser degree RA-7, induced hypothermia in 5-HT$_7^{+/+}$ but not in 5-HT$_7^{-/-}$ mice. Our results suggest that LP-211 can be used as a 5-HT$_7$ receptor agonist *in vivo*.

Keywords

serotonin 5-HT$_7$ receptor; agonist; LP-211; RA-7; brain distribution; body temperature

The 5-HT$_7$ receptor has been cloned from mouse, rat, guinea-pig, pig and human and the binding profile is consistent across species and between cloned and native receptors [1–3]. 5-HT$_7$ receptors are defined pharmacologically by their high affinity for 5-CT (5-carboxamidotryptamine), 5-HT (5-hydroxytryptamine), 5-MeOT (5-methoxytryptamine) and methiothepin, moderate affinity for 8-OH-DPAT (8-hydroxy-*N*,*N*-dipropylaminotetraline) and ritanserin and low affinity for pindolol, sumatriptan and buspirone [4].

In brain tissue, 5-HT$_7$ receptor mRNA is localized within the thalamus, hypothalamus, limbic and cortical regions [4–6]. Neurochemical lesioning indicate that, at least in the hypothalamus, they are located post-synaptically [7]. Autoradiography has shown a pattern

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of 5-HT$_7$ binding sites in the brain [4,8,9] which matches, to a large extent, the mRNA distribution.

Studies using selective antagonists such as SB-258719 ((R)-3,N-dimethyl-N-[1-methyl-3-(4-methylpiperidin-1-yl)propyl]benzene sulfonamide) and SB-269970 ((2R)-1-[(3-hydroxyphenyl)sulfonyl]-2-[2-(4-methyl-1-piperidinyl)ethyl]pyrrolidine), as well as mice lacking the receptor, have suggested that targeting 5-HT$_7$ receptors may have therapeutic applications in depression [3] and may play a role in cognitive processes [10,11].

In recent years considerable efforts have been focused on the development of selective 5-HT$_7$ receptor agonists and antagonists [12,13]. The first selective antagonist, SB-258719, was identified in 1997 by GlaxoSmithKline, who subsequently identified SB-269970 that is considered to date the standard selective 5-HT$_7$ receptor antagonist [14]. However, further studies with SB-269970 have been hampered by its rapid clearance *in vivo*. The antagonist SB-656104 ((6-((R)-2-[2-[4-(4-chloro-phenoxy)-piperidin-1-yl]-ethyl]-pyrrolidine-1-sulphonyl)-1H-indole) showed an improved pharmacokinetic profile, but displayed modest selectivity over 5-HT$_{2A}$, 5-HT$_{2B}$, and 5-HT$_{1D}$ receptors [15]. On the agonists side, the mixed 5-HT$_{1A}$/5-HT$_7$ ligand 8-OH-DPAT has been used to activate 5-HT$_7$ receptors in the presence of the 5-HT$_{1A}$ receptor antagonist WAY 100635 (N-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-N-(2-pyridyl)cyclohexanecarboxamide) [10]. Recently characterization of the selective 5-HT$_7$ agonists AS-19 ((2S)-(+)-5-(1,3,5-trimethylpyrazol-4-yl)-2-(dimethylamino)tetralin) and E-55888 (dimethyl-{2-[3-(1,3,5-trimethyl-1H-pyrazol-4-yl)-phenyl]-ethyl}-amine dihydrochloride) has been reported [16]. We have recently developed a series of arylpiperazine derivatives as potential 5-HT$_7$ receptor agonists [17]. Among these N-(4-cyanophenylmethyl)-4-(2-diphenyl)-1-piperazinehexanamide (LP-211, identified as compound 25 in [17]) displayed potent 5-HT$_7$ receptor agonist activity while maintaining relatively low affinity for 5-HT$_{1A}$ and dopamine D$_2$ receptors. In the present study we report on the *in vitro* and *in vivo* activity profile of LP-211 by studying radioligand binding, body temperature regulation in mice lacking the 5-HT$_7$ receptor (5-HT$_7^{-/-}$) and their 5-HT$_7^{+/+}$ sibling controls, and pharmacokinetic disposition in these mice as well as in rats. Also, a similar pharmacological characterization was carried out on 1-(2-diphenyl)piperazine (RA-7), the main metabolite of LP-211.

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Competition binding assays were performed using transfected or stably expressing cell membrane preparations as previously described [18]. Radioligand identity, radioligand concentration, and K_i values of reference compounds are listed in Table 1. Incubation buffer was 50 mM Tris HCl, 10 mM MgCl$_2$, 0.1 mM EDTA, pH 7.4 in all cases, except SERT where 50 mM Tris HCl, 150 mM NaCl, 5 mM KCl, pH 7.4 was used. In all cases the incubation time was 1.5 h. Additional information is available online (http://pdsp. med.unc.edu/UNC-CH%20Protocol%20Book.pdf).

The effect of LP-211 and RA-7 on body temperature was studied using ten-to-twelve week old male 5-HT$_7^{-/-}$ mice with their male 5-HT$_7^{+/+}$ siblings used as controls. The generation of the 5-HT$_7^{-/-}$ mouse strain has been described previously [19]. The experiments were performed at The Scripps Research Institute (TSRI) where the mice were housed in a 12-hour light/dark cycle (lights on at 06.00 and off at 18.00) and had free access to water and food pellets. The experiments were done in accordance with the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the US National Institutes of Health,

and were approved by the Animal Care and Use Committee at TSRI. Every effort was made to reduce the number of animals used and to minimize potential suffering.

LP-211 (as tartrate salt), RA-7 (as hydrochloride salt) (both synthesized at the Dipartimento Farmaco-Chimico, Università degli Studi di Bari), WAY 100135 ((S)-N-tert-butyl-3-(4-(2-methoxyphenyl)-piperazin-1-yl)-2-phenylpropanamide), or SB-269970 were given as single intraperitoneal injections in a volume of 0.2 ml. WAY 100135 and SB-269970 were dissolved in 0.9% saline and administered 30 min prior to LP-211. LP-211 and RA-7 were dissolved in 100% ethanol to a 10× concentration and then diluted in saline immediately prior to injection, and 10% ethanol in saline was used as control. The doses used in this study were chosen based on published data [20,19,21].

Core body temperature was measured using a rectal thermometer probe as previously described [19,21]. A basal value was measured immediately before injections and then measurements were made 15 minutes after the injection and subsequently every 30 minutes for two hours. All experiments were started at 09.00 h to control for circadian variability in body temperature.

In disposition experiments different groups of $5\text{-}HT_7^{+/+}$ and $5\text{-}HT_7^{-/-}$ mice were given LP-211 as above. Thirty minutes after injection, the mice were anaesthetized with an isoflurane overdose. Trunk blood was collected and allowed to coagulate. The brain was removed and stored at $-80\ °C$ until analysis. The blood was centrifuged and the serum was stored at $-80\ °C$ until analysis.

Compounds LP-211 and RA-7 were extracted from plasma and brain homogenate (distilled water, $1\ g \cdot 10\ mL^{-1}$) and quantified by reversed-phase high-performance liquid chromatography with UV detection (230 nm), with slight modifications to the procedures previously described [17]. Analytes were identified on the basis of their retention time, 8.6 min for LP-211, 6.4 min for RA-7 and 10.9 min for the internal standard. Coefficient of variation (C.V.) for the precision and reproducibility of the assay at the lower limit of quantification ($50\ ng \cdot mL^{-1}$ or g^{-1}) was 15–20%. Higher concentrations gave C.V. generally less than 10%.

Values reported in Tables 1 and 2 and Figure 1 are expressed as means ± standard errors of mean (S.E.M.). Possible differences between genotypes and/or drug treatments were analyzed using two-way analysis of variance (ANOVA) with genotype as one variable and drug treatment as the other variable. The ANOVA was followed by an appropriate Bonferroni posttest. Differences were considered significant at P < 0.05.

The radioligand binding screening confirmed that LP-211 bound to $5\text{-}HT_7$ receptors with high affinity ($K_i = 15\ nM$). LP-211 was also at least 5-fold selective over a range of 5-HT receptor subtypes, and 54-fold selective over SERT. RA-7 also displayed high affinity for the $5\text{-}HT_7$ receptor ($K_i = 1.4\ nM$) and was > 54-fold selective over a range of 5-HT receptor subtypes and SERT (Table 1).

There was no difference in basal body temperature between $5\text{-}HT_7^{+/+}$ and $5\text{-}HT_7^{-/-}$ mice (Table 2), and vehicle-treated animals maintained a stable body temperature which fluctuated by no more than $0.5\ °C$ over the 2h recording period, regardless of genetic status (Fig. 1). LP-211 (3, 10, and $30\ mg \cdot kg^{-1}$, i.p.) dose-dependently reduced body temperature in wild-type mice with a peak effect at 15–30 min after the injection (Fig. 1). For the doses tested body temperature returned to base level within the registration period. Statistically, the effects of LP-211 and RA-7 were evaluated as peak change and as overall change (Table 2). The lowest dose of LP-211 ($3\ mg \cdot kg^{-1}$) tested did not alter body temperature in either $5\ HT_7^{+/+}$ or $5\ HT_7^{-/-}$ mice. At $10\ mg \cdot kg^{-1}$ LP-211 had a peak effect in $5\text{-}HT_7^{+/+}$ mice, but

not in 5-HT$_7^{-/-}$ mice. There was also an overall hypothermic effect in the 5 HT$_7^{+/+}$ mice. At the highest dose tested (30 mg · kg^{-1}), LP-211 had a significant effect in 5-HT$_7^{+/+}$ mice both when analyzing peak change and overall change. At this dose (30 mg · kg^{-1}), LP-211 also had a significant, but less pronounced, effect on peak change in 5-HT$_7^{-/-}$ mice.

The 5-HT$_7$ receptor antagonist SB-269970 (10 mg · kg^{-1}, i.p.) blocked the effect of LP-211 (30 mg · kg^{-1}) on body temperature (Fig. 1E, Table 2). SB-269970 itself had no effect on body temperature. The 5-HT$_{1A}$ receptor antagonist WAY 100135 (10 mg · kg^{-1}, i.p.) failed to counteract the hypothermic effect of LP-211 (Fig. 1F); it rather appeared to unmask other effects on body temperature, the cause of which remains to be determined.

The effect of RA-7 on body temperature was tested for 3 and 10 mg · kg^{-1} (Fig. 1B, C; Table 2). Interestingly, at 3 mg · kg^{-1} RA-7 had a slight but significant hyperthermic effect. At 10 mg · kg^{-1} RA-7 induced hypothermia to small degree that reached significance for overall change in the 5 HT$_7^{+/+}$ mice.

It should be noted that, apart from the hypothermia, LP-211 or RA-7 did not induce any visible adverse effects in the mice.

Other groups of 5-HT$_7^{-/-}$ and 5-HT$_7^{+/+}$ mice were given 10 and 30 mg · kg^{-1} LP-211 i.p. and were killed 30 min thereafter to obtain basic information on the parent compound and metabolite concentrations achieved in plasma and brain. Plasma concentrations of LP-211 at approximately the time of peak effect on body temperature were essentially dose-related, ranging from 2.1 ± 0.3 and 1.8 ± 0.3 nmol · mL^{-1} at 10 mg · kg^{-1} to 4.5 ± 0.7 and 4.1 ± 0.5 at 30 mg · kg^{-1} in 5-HT$_7^{-/-}$ and 5-HT$_7^{+/+}$ mice, respectively. Brain concentrations (Fig. 2A) reflected plasma concentrations with mean brain-to-plasma distribution ratios around 0.3–0.4, regardless of genotype. As shown in Fig. 2B there was a linear relationship between plasma and brain concentrations 30 min after i.p. doses of 10–30 mg · kg^{-1} in these mice (brain = −291 + 0.61 plasma; r^2 = 0.78), indicating that LP-211 passes through the blood-brain barrier by passive diffusion and that plasma concentrations accurately predict whole brain concentrations.

The metabolite RA-7 was undetectable in plasma and brain of 5-HT$_7^{-/-}$ and 5-HT$_7^{+/+}$ mice 30 min after the lowest dose used in the LP-211-induced hypothermia test. It became detectable in the samples of some mice (n = 2–3 per group) at 30 mg · kg^{-1}. At this time mean metabolite plasma concentrations approached only about 3–4% of LP-211 plasma concentrations, on a molar basis. Mean brain concentrations (about 0.3 nmol ·g^{-1} in both groups) were higher than plasma concentrations, in agreement with previous reports of concentrations of this [17] and other arylpiperazine derivatives in rodent brain [22]. Therefore, the metabolite-to-parent drug ratio was slightly higher in brain (about 0.5, on a molar basis) than in plasma, although varied largely among animals of both groups.

The compound LP-211 has recently been identified as a 5-HT$_7$ receptor agonist [17]. It showed a K_i value of 0.58 nM at rat cloned 5-HT$_7$ receptors, and good selectivity (>300-fold) over the 5-HT$_{1A}$ receptor. LP-211 was characterized as a competitive agonist at the 5-HT$_7$ receptor by measuring relaxation of substance P induced contraction in an isolated guinea pig ileum assay [17]. LP-211 behaved as a full agonist (82% maximal activity compared to 5-CT) with an EC$_{50}$ value of 0.60 μM which is close to that of 5-CT (EC$_{50}$ = 0.63 μM). The observed effect was reversed by the selective 5-HT$_7$ receptor antagonist SB-269970. Disposition studies in mouse demonstrated that LP-211 rapidly entered the brain achieving whole-brain concentrations comparable to those in plasma. Like arylpiperazine derivatives in general [22], metabolism of LP-211 included *N*-dealkylation of the aliphatic chain attached to the piperazine nitrogen, resulting in the formation of 1-(2-diphenyl)piperazine (RA-7). This potentially active metabolite concentrated in mouse brain,

yielding a mean metabolite-to-parent compound ratio of about 2 on a molar basis. Other 1-arylpiperazine's metabolites have similar pharmacokinetic behavior in rodents, concentrating in the brain more than their parent drug(s) and possibly contributing to or accounting for some of their central effects [22]. All these data prompted us to investigate further the *in vitro* binding profile of LP-211 and also its activity profile in a simple test (body temperature regulation) in mice lacking the 5-HT$_7$ receptor (5-HT$_7^{-/-}$) and their 5-HT$_7^{+/+}$ sibling controls. The same evaluations were also performed on RA-7 to assess its possible contribution to the *in vivo* activity of LP-211.

As illustrated in Table 1, LP-211 showed a K_i value of 15 nM at human cloned 5-HT$_7$ receptors, which is 25-fold greater than the K_i value previously found at rat cloned 5-HT$_7$ receptor ($K_i = 0.58$ nM) [17]. The difference could be due to differences between receptors cloned from different species or to different binding protocols. Moreover, the affinity profile of LP-211 seems preferable compared to that of 8-OH-DPAT which has been reported to have $K_i = 467$ nM at the human cloned 5-HT$_7$ receptor and $K_i = 3.8$ nM at human cloned 5-HT$_{1A}$ [23]. The selectivity of LP-211 against 5-HT$_{1B}$, 5-HT$_{2B}$, 5-HT$_{2C}$ and 5-HT$_{5A}$ was modest (5-14-fold), whereas significantly higher selectivities were observed for the subtypes 5-HT$_{1A}$ (25-fold), 5-HT$_{1D}$ (26-fold), 5-HT$_{2A}$ (40-fold) and 5-HT$_6$ (105-fold). The selectivity observed at 5-HT$_{1A}$ receptor might, at least in most cases, be sufficient to avoid interference from biological response mediated by this receptor *in vivo*. Finally, LP-211 did not significantly bind at 5-HT$_3$ and 5-HT$_{1E}$ receptors, or at SERT. Interestingly, RA-7 appeared to be a potent ligand for the human 5-HT$_7$ receptor with a K_i (1.4 nM), approximately 10-fold lower than the corresponding value for LP-221. Overall, RA-7 was more specific than LP-211 *in vitro*, mainly because RA-7 showed higher affinity for the 5-HT$_7$ receptor than LP-211. Moreover, RA-7 was less than 100-fold selective only over 5-HT$_{5A}$ and 5-HT$_{1A}$ receptors (54- and 71-fold).

Compound LP-211 seemed to dose-dependently reduce body temperature in 5-HT$_7^{+/+}$ mice but not in 5-HT$_7^{-/-}$ mice when analyzing both peak and overall effect. At the higher dose tested, body temperature was reduced also in 5-HT$_7^{-/-}$ mice when analyzing peak effect, but not when analyzing overall effect. The peak effect in 5-HT$_7^{-/-}$ mice can most likely be attributed to an action at 5-HT$_{1A}$ receptors [21]. The 5-HT$_{1A}$ receptor antagonist WAY 100135 failed to block the hypothermic effect of LP-211. WAY 100135 rather appeared to unmask other effects on body temperature, the cause of which remains to be determined. It should however be noted that WAY 100135 is a partial agonist at 5-HT$_{1A}$ receptors which will also lower body temperature when activated [21,24]. The 5-HT$_7$ receptor antagonist SB-269970 blocked the effect of LP-211 on body temperature as the effects seen when combining LP-211 and SB-269970 were not different from vehicle. Taking into account the pharmacokinetic results showing that plasma and brain concentrations of LP-211 and its active metabolite RA-7 at approximately the time of peak effect on body temperature were essentially similar in either wild-type and knockout mice, it can be concluded that the hypothermic effect of LP-211, at least primarily, is mediated by the 5-HT$_7$ receptor. In support it should be noted that the ability of LP-211 to induce hypothermia was similar to that seen for the unselective agonists 5-HT, 5-CT, and 8-OH-DPAT, but possibly with an enhanced ability for SB-269970 to inhibit the response [19,21].

Interestingly, the metabolite RA-7 also affected body temperature, but for most parts in a way that was not different between 5-HT$_7^{+/+}$ and 5-HT$_7^{-/-}$ mice. This observation stands in contrast to the apparent 5-HT$_7$ receptor selectivity seen for RA-7 in the binding profile. As mentioned, RA-7 rapidly entered the brain achieving higher concentrations than in plasma.

Based on the affinity profiles, it cannot be excluded that at higher doses, LP-211 and RA-7 act also on 5-HT$_{1A}$ receptors to induce hypothermia [21]. This is consistent with the finding

that WAY 100135, a partial agonist, failed to counteract the effect of LP-211. Further studies are needed to fully determine the mechanisms at play.

In conclusion, our quest to produce new ligands for the serotonin 5-HT$_7$ receptor has resulted in the development of the 5-HT$_7$ receptor agonist LP-211, which shows suitable pharmacodynamic and pharmacokinetic properties for *in vivo* studies aimed to characterize the functional role of this receptor. Continued work comparing LP-211 with other recently described 5-HT$_7$ receptor agonists such as AS-19 and E-55888 is needed to fully establish their actual *in vivo* usability.

Acknowledgments

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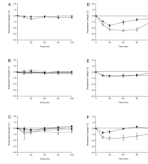
Fig. 1.
Effects of LP-211 (square symbols) and RA-7 (round symbols) on body temperature in 5-HT$_7^{+/+}$ (open symbols) and 5-HT$_7^{-/-}$ mice (filled symbols). (A) vehicle (10% ethanol in saline); (B) LP-211 and RA-7, 3 mg · kg^{-1} i.p.; (C) LP-211 and RA-7, 10 mg · kg^{-1} i.p.; (D) LP-211, 30 mg · kg^{-1} i.p.; (E) LP-211 30 mg · kg^{-1} + SB-269970 10 mg · kg^{-1} i.p.; (F) LP-211 30 mg · kg^{-1} + WAY 100135 10 mg · kg^{-1} i.p. For basal values and statistical evaluation see Table 2.
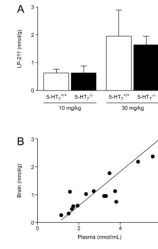
Fig. 2.
Mean brain concentrations of LP-211 (A) and the relationship (individual animals) between brain and plasma concentrations (B) after intraperitoneal doses in 5-HT$_7^{-/-}$ and 5-HT$_7^{+/+}$ mice. Values are mean ± S.D. for 5 mice per treatment group.

Table 1

K_i estimates for LP-211 and RA-7 at a panel of human 5-HT receptors and at human SERT

Target	K_i (nM)		Radioligand	Reference Compound (K_i, nM)
	LP-211	**RA-7**		
h5-HT$_7$	15 ± 1	1.4 ± 0.2	1.1 nM [^{3}H]LSD	Chlorpromazine (64)
h5-HT$_{1A}$	379 ± 118	99 ± 22	0.5 nM [^{3}H]8-OH-DPAT	Methysergide (38)
h5-HT$_{1B}$	215 ± 10	1021 ± 121	0.29 nM [^{3}H]GR125743	Ergotamine (2.7)
h5-HT$_{1D}$	394 ± 74	302 ± 39	1.4 nM [^{3}H]5-CT	Ergotamine (4.7)
h5-ht$_{1E}$	>10,000	>10,000	3 nM [^{3}H]5-HT	5-HT (7.5)
h5-HT$_{2A}$	626 ± 141	1190 ± 288	0.5 nM [^{3}H]ketanserin	Chlorpromazine (9.6)
h5-HT$_{2B}$	67 ± 10	178 ± 19	1.0 nM [^{3}H]LSD	Methysergide (2.4)
r5-HT$_{2C}$	91 ± 9	171 ± 14	0.5 nM [^{3}H]mesulergine	Chlorpromazine (22)
h5-HT$_3$	>10,000	228 ± 34	0.29 nM [^{3}H]LY278584	LY278584 (1.7)
h5-ht$_{5A}$	178 ± 26	76 ± 14	0.99 nM [^{3}H]LSD	Ergotamine (7)
h5-HT$_6$	1571 ± 193	596 ± 70	0.97 nM [^{3}H]LSD	Chlorpromazine (32)
hSERT	812 ± 156	439 ± 41	0.79 nM [^{3}H]citalopram	Amytriptyline (9.3)

Table 2

Effects of LP-211 and its main metabolite RA-7 on body temperature in 5-HT$_7$$^{+/+}$ and 5-HT$_7$$^{-/-}$ mice.

| Treatment | 5-HT$_7$$^{+/+}$ | | | | 5-HT$_7$$^{-/-}$ | | | |
	n	Basal value (°C)	Changes Peak (%)	Overall (arbitrary units)	n	Basal value (°C)	Changes Peak (%)	Overall (arbitrary units)
Vehicle	14	34.91 ± 0.09	−1.44 ± 0.33	−21.83 ± 9.92	14	35.11 ± 0.07	−1.00 ± 0.23	−22.53 ± 6.73
LP-211 (3 mg/kg)	6	35.01 ± 0.04	−0.76 ± 0.21	−17.48 ± 5.14	6	34.98 ± 0.12	−0.48 ± 0.30	0.12 ± 11.43
LP-211 (10 mg/kg)	8	35.03 ± 0.22	−2.82 ± 1.03 ***	−73.58 ± 27.99 *	8	35.03 ± 0.15	−1.63 ± 0.84 †	−31.08 ± 15.84
LP-211 (30 mg/kg)	8	34.99 ± 0.04	−5.10 ± 0.80 ***	−124.22 ± 33.58 ***	8	34.83 ± 0.12	−1.85 ± 0.66 *†††	−18.04 ± 14.86 †††
WAY-100135 (10 mg/kg)	5	35.02 ± 0.10	−0.19 ± 0.34	N/D	5	34.96 ± 0.04	−0.31 ± 0.32	N/D
LP-211 (30 mg/kg) + WAY-100135 (10 mg/kg)	5	34.95 ± 0.06	−6.45 ± 0.65 ***	−211.62 ± 23.02 ***	5	34.85 ± 0.08	−4.54 ± 0.62 ***	−104.76 ± 21.54 ***†††
SB-269970 (10 mg/kg)	5	35.04 ± 0.04	−0.10 ± 0.15	N/D	5	35.00 ± 0.05	0.06 ± 0.21	N/D
LP-211 (30 mg/kg) + SB-269970 (10 mg/kg)	5	35.00 ± 0.02	−2.21 ± 0.17	−61.5 ± 7.92	5	35.02 ± 0.03	−1.84 ± 0.18	−62.57 ± 7.87
RA-7 (3 mg/kg)	6	34.97 ± 0.08	1.12 ± 0.16 ***	2.72 ± 8.41	6	34.99 ± 0.09	0.64 ± 0.13 *	−9.28 ± 8.28
RA-7 (10 mg/kg)	11	35.26 ± 0.15	−2.13 ± 0.49	−65.88 ± 16.31 ***	10	35.24 ± 0.10	−0.87 ± 0.54	−23.79 ± 12.17

Changes are expressed as peak (maximum effect during the first 30 minutes after injection) and overall (area under the curve) effects for the 2-hour registration period.

*
P < 0.05,

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P < 0.01,

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P < 0.001 two-way analysis of variance followed by Bonferroni posttests with respect to corresponding vehicle control.

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P < 0.05,

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P < 0.01,

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P < 0.001 two-way analysis of variance followed by Bonferroni posttests comparing 5-HT$_7$$^{+/+}$ and 5-HT$_7$$^{-/-}$ mice receiving the same treatment.