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Genotype-Dependent Activity of Tryptophan Hydroxylase-2 Determines the Response to Citalopram in a Mouse Model of Depression

Luigi Cervo, Alessandro Canetta, Eleonora Calcagno, Silvia Burbassi, Giuseppina Sacchetti, Silvio Caccia, Claudia Fracasso, Diego Albani, Gianluigi Forloni, and Roberto W. Invernizzi

Istituto di Ricerche Farmacologiche Mario Negri, Department of Neuroscience, 20157 Milan, Italy

Polymorphism of tryptophan hydroxylase, the rate-limiting enzyme in the synthesis of brain serotonin (5-HT), is associated with less synthesis of brain 5-HT in DBA/2J and BALB/c than in C57BL/6J and 129/Sv mice. We selected the forced swimming test, a mouse model used to assess the antidepressant potential of drugs, and neurochemical techniques to study strain differences in the response to citalopram, a selective 5-HT reuptake inhibitor. Citalopram reduced immobility time in C57BL/6J and 129/Sv mice but had no such effect in DBA/2J and BALB/c mice. The drug reduced accumulation of 5-hydroxytryptophan (5-HTP), an indicator of 5-HT synthesis, in C57BL/6J and 129/Sv mice but much less in DBA/2J and BALB/c mice. Pretreatment with tryptophan raised 5-HTP accumulation and reinstated the antidepressant-like effect of citalopram in DBA/2J and BALB/c mice, whereas pharmacological inhibition of 5-HT synthesis prevented the effect of citalopram in C57BL/6J and 129/Sv mice. Because there were no strain differences in catecholamine synthesis, locomotor activity, and brain levels of citalopram at the end of the behavioral test, the results suggest that the failure of citalopram to reduce immobility time in DBA/2J and BALB/c mice is attributable to genotype-dependent impairment of 5-HT synthesis. Interstrain comparisons could probably be a useful strategy for understanding the mechanisms underlying the response to selective serotonin reuptake inhibitors.

Key words: antidepressant; serotonin; tryptophan hydroxylase-2; genetic polymorphism; 5-HT synthesis; SSRIs

Introduction

Antidepressant drugs have greatly improved the outcome of depression. However, a considerable proportion of patients show only a partial or no response, regardless of the treatment received (Thase and Rush, 1995; Stimpson et al., 2002; Fava, 2003). Selective serotonin (5-HT) reuptake inhibitors (SSRIs) are currently the mainstays in the treatment of depression. Pharmacogenetic studies have linked the response to SSRIs to polymorphism in various genes such as those responsible for reuptake, biosynthesis, and metabolism of 5-HT and genes coding for some 5-HT receptor subtypes and signal transduction mechanisms (Serretti and Artioli, 2004).

Tryptophan hydroxylase (TPH)-2, a new isoform of TPH, the rate-limiting enzyme in the biosynthesis of 5-HT, was recently discovered preferentially located in the brain, whereas TPH-1 (formerly TPH) is mainly located in other tissues (Walther et al., 2003). A single nucleotide polymorphism (SNP) of human TPH-2 (G1463A), replacing Arg 441 with His, has been identified (Zhang et al., 2005). Mutation of this gene resulted in a marked

reduction of 5-HT production when the enzyme was expressed in PC12 cells. In addition, the mutant allele in elderly patients with unipolar major depression (10%) was significantly more frequent than in nondepressed controls (1.4%) and was associated with a poor response to SSRIs (Zhang et al., 2005).

The gene encoding for TPH-2 shows an SNP (C1473G) that results in the substitution of Pro[447] with Arg[447], with different allelic distribution in different inbred strains of mice (Zhang et al., 2004). DBA/2J and BALB/c mice, homozygous for the allele 1473G, have a lower 5-HT synthesis rate than C57BL/6J and 129/Sv mice, homozygous for the allele 1473C (Zhang et al., 2004). Therefore, comparison of the effects of SSRIs in these strains may offer a valid animal model to investigate the neurochemical bases of the response to SSRIs.

Several studies have reported marked strain differences in the response to SSRIs in the forced swimming test (FST) (Lucki et al., 2001; David et al., 2003), a procedure widely used to assess the antidepressant potential of drugs in rats and mice (Porsolt et al., 1977, 1978). However, these studies have given conflicting results, and no firm conclusion can be drawn about the relationship between genotype-dependent differences in 5-HT synthesis and the response to SSRIs.

To address this, we compared the immobility time in the FST in C57BL/6J, 129/Sv, DBA/2J and BALB/c mice under basal conditions and in response to citalopram, the most selective inhibitor of the 5-HT transporter molecule among the SSRIs (Hyttel, 1977;

Pozzi et al., 1999; Pollock, 2001). Next, we assessed the influence of strain and citalopram on brain 5-HT and catecholamine synthesis rates by measuring the accumulation of 5-hydroxytryptophan (5-HTP) and dihydroxyphenylalanine (DOPA), the immediate precursors of 5-HT and catecholamines, after inhibition of aromatic L-amino acid decarboxylase with m-hydroxybenzylhydrazine (Carlsson et al., 1972; Broadhurst and Briley, 1988). Finally, mice were given the 5-HT precursor L-tryptophan (TRP) or the 5-HT synthesis inhibitor p-chlorophenylalanine (PCPA) (Koe and Weissman, 1966) to assess how treatments that boost or lower 5-HT synthesis affect the citalopram response.

Materials and Methods

Animals
Male C57BL/6J, 129/Sv, DBA/2J, and BALB/c mice (Charles River, Calco, Italy), 6–8 weeks old, were housed five per cage at constant room temperature ($21 \pm 1°C$) and relative humidity (60%) under a regular light/dark schedule (light on from 8:00 A.M. to 8:00 P.M.) with food and water available *ad libitum*. The animals were allowed to adapt to laboratory conditions for at least 1 week. All mice were used only once. Experiments were performed in the daylight, between 10:00 A.M. and 1:00 P.M.

Procedures involving animals and their care were conducted in conformity with the institutional guidelines that are in compliance with national (Decreto Legge number 116, Gazzetta Ufficiale, supplement 40, February 18, 1992; Circolare number 8, Gazzetta Ufficiale, June 14, 1994) and international laws and policies (EEC Council Directive 86/609; NRC, 2003).

Genotyping and PCR conditions
Genomic DNA was purified from tail snips using a commercial kit (Promega, Milan, Italy). To assess strain genotype, a PCR reaction was done as follows: 94°C, 5 min (one time), 94°C, 30 s; 62°C, 30 s; 72°C, 30 s (40 times), 72°C, 5 min (one time). The following primers were used (Zhang et al., 2004): forward, 5′-TTTGACCCAAAGACGACCTGCTTGCA; reverse C-allele specific, 5′-CAGAATTTCAATGCTCTGGTGGG; and reverse G-allele specific, 5′-CAGAATTTCAATGCTCTGGTGGC. For each strain, PCR was done as described using the same forward primer and either the C-allele- or G-allele-specific reverse primer. The expected size of the amplified band was 307 bp. PCR products were loaded on 1% agarose gel and visualized using ethidium bromide staining. All samples were also checked for DNA integrity by PCR amplification of β-actin gene.

Immobility and locomotor activity
Forced swimming. The FST was essentially similar to that described previously (Renard et al., 2004). Mice were placed individually in clear Plexiglas cylinders (height, 25 cm; diameter, 10 cm) containing 15 cm of water at $25 \pm 1°C$, and their behavior was videotaped for 6 min. The total period of immobility during the last 4 min was timed later by two observers who did not know which treatment the mice had received. A mouse was judged to be immobile when it remained floating, in an upright position, making only small movements to keep its head above water. In one experiment, the response to citalopram of C57BL/6J and DBA/2J mice was evaluated in a 20-cm-wide cylinder (height, 40 cm) containing 15 cm of water at $25 \pm 1°C$.

Open field. Separate groups of mice were used to see how treatments reducing immobility in the FST affected locomotor activity. Mice were given citalopram alone or with TRP, as described below (see Drugs and treatments). Thirty minutes after citalopram, they were placed individually in an open field made of gray plastic (40×40 cm) with the floor divided into 25 equal squares. Spontaneous locomotor activity was measured by counting the number of squares crossed in a 4 min period, corresponding to the behavioral observation time in the FST.

Citalopram measurement
At the end of the pharmacological test, animals were killed by decapitation, and trunk blood was collected in heparinized tubes and stored at 4°C for 1 h. The brain was rapidly removed and stored at −20°C until

analysis. Plasma was prepared by centrifugation ($2000 \times g$ for 10 min, at 4°C). Citalopram and its main metabolite N-demethylcitalopram were determined in plasma and brain tissue by HPLC with UV detection (235 nm), according to Grignaschi et al. (1998). The lower limits for quantification (LOQ) were 0.05 μg/ml and 0.08 μg/g, using 250 μl of plasma or ~200 mg of brain tissue.

Neurochemical studies
The brain was rapidly excised from the skull and cut along the sagittal line. Half of the brain was immediately frozen on dry ice. The striatum was dissected out from the other half and frozen. Tissue samples were stored at −80°C until analysis. Tissues were homogenized by sonication (output, two to three; 30–35 pulses with a Branson Sonifier, model 250; Branson Ultrasonic Corporation, Danbury, CT) in 10 vol of ice-cold 0.1 M HClO$_4$. After centrifugation ($4800 \times g$ for 10 min, at 4°C), the clear supernatant was injected into the HPLC equipped with a 150×3.9 mm C18 reverse-phase column (Nova-pack; Waters) and coupled to an electrochemical detector (Coulochem II; ESA, Chelmsford, MA). 5-HTP and DOPA were determined in the same sample according to the procedure described previously (Lasley et al., 1984). Brain 5-HT concentrations were determined in separate samples using the same analytical procedure.

Drugs and treatments
Citalopram hydrobromide (Tocris Cookson, Bristol, UK), dissolved in sterile distilled water, or vehicle was injected intraperitoneally 30 min before the 6 min FST test. A group of DBA/2J and BALB/c mice pretreated intraperitoneally with 300 mg/kg TRP (Fluka, Buchs, Switzerland) or vehicle was injected with citalopram (5 mg/kg) or vehicle 30 min later, and immobility in the FST was evaluated 30 min after the last injection. A group of C57BL/6J and 129/Sv mice was given 100 mg/kg PCPA (Sigma-Aldrich, Milan, Italy) or vehicle orally for 3 consecutive days. Twenty-four hours after the last dose, they were given 5 mg/kg citalopram or vehicle, and, 30 min later, their immobility was evaluated in the FST. Brain and plasma levels of citalopram and its main metabolite were measured in the same mice at the end of the behavioral test.

Separate groups of mice were used to evaluate the effects of treatments on 5-HTP and DOPA accumulation after inhibition of aromatic L-amino acid decarboxylase with m-hydroxybenzylhydrazine (Carlsson and Lindqvist, 1978), injected (100 mg/kg, i.p.) 0.5 h after citalopram; mice were killed by decapitation 30 min later.

The solutions were freshly prepared immediately before use and injected in a volume of 10 ml/kg. Doses of citalopram and m-hydroxybenzylhydrazine were referred to the respective salts. Doses of PCPA and TRP were calculated as free bases.

Statistics
To determine whether basal immobility time, locomotor activity, accumulation of brain 5-HTP and DOPA, and brain levels of 5-HT differed between strains, data were analyzed by one-way ANOVA, followed by Tukey-Kramer's test or by Student's *t* test. The effects of vehicle and 2.5 and 5 mg/kg citalopram on immobility time and the effects of vehicle and 5 mg/kg citalopram on 5-HTP and DOPA accumulation in different strains were analyzed by two-way ANOVA, with strain and treatment as main factors. Likewise, brain and plasma levels of citalopram in different strains of mice given 2.5 and 5 mg/kg of the drug were compared by two-way ANOVA, with strain and treatment as main factors. The effect of citalopram on immobility time in each strain was compared with that vehicle by one-way ANOVA. *Post hoc* comparisons between means were made by Tukey-Kramer's test.

Results
Figure 1 shows the C1473G polymorphism of TPH-2 and its different allelic distribution in C57BL/6J, 129/Sv, DBA/2J, and BALB/c mice. In agreement with previous results, C57BL/6J and 129/Sv mice are homozygous for the allele C and DBA/2J and BALB/c mice for the allele G. PCR amplification of the β-actin gene confirmed that DNA was not damaged (data not shown).
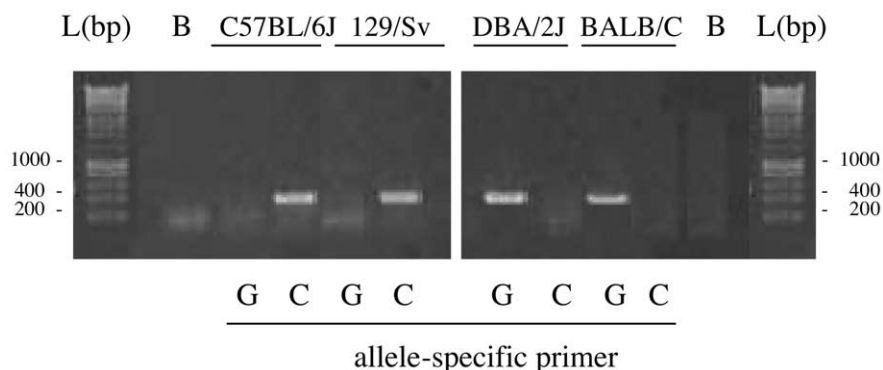
L(bp)　B　C57BL/6J　129/Sv　DBA/2J　BALB/C　B　L(bp)

Figure 1. Genotypic analysis of C57BL/6J, 129/Sv, DBA/2J, and BALB/c mice strains to assess C1473G polymorphism in the mTph2 gene shows that C57BL/6J and 129/Sv strains have the C allele, and DBA/2J and BALB/c strains have the G allele. L, DNA ladder; B, PCR control sample.

Table 1. Immobility time (pool of two experiments) of mice treated with vehicle

Strain	Immobility time (s)
C57BL/6J	170.9 ± 4.7 ($n=26$)
129/Sv	204.6 ± 4.4*($n=20$)
DBA/2J	159.8 ± 6.8 ($n=22$)
BALB/c	171.8 ± 5.1 ($n=20$)

Data are means ± SEM; the number of mice in each group is indicated in parentheses. *$p < 0.05$ versus C57BL/6J, DBA/2J, and BALB/c

Effect of citalopram on immobility time and open-field activity

Immobility time

Because no significant differences were found between experiments in basal immobility time regardless of the number of vehicle injections (C57BL/6J, $t_{(24)} = 1.1$, $p = 0.3$; 129/Sv, $t_{(18)} = 0.7$, $p = 0.5$; DBA/2J, $t_{(20)} = 0.3$, $p = 0.8$; BALB/c, $t_{(18)} = 1.2$, $p = 0.3$), data from each strain were pooled. As reported in Table 1, there was a significant difference in the basal immobility time between strains ($F_{(3,84)} = 12.3$; $p < 0.0001$). Immobility time in 129/Sv mice was significantly longer than the other strains ($p < 0.05$, Tukey-Kramer's test). No significant differences were found in basal immobility between C57BL/6J, DBA/2J, and BALB/c mice.

Marked differences were found in the responses of the four strains to citalopram in the FST (Fig. 2). Overall, two-way ANOVA indicates a significant effect of strain ($F_{(2,106)} = 14.9$; $p < 0.0001$), treatment ($F_{(3,106)} = 43.6$; $p < 0.0001$), and strain × treatment interaction ($F_{(6,106)} = 12.8$; $p < 0.0001$). Citalopram dose dependently reduced immobility time in C57BL/6J mice ($F_{(3,35)} = 18.2$; $p < 0.01$). The effect was significant at 2.5 and 5 mg/kg, whereas 1.25 mg/kg had no effect. Doses of 2.5, 5, and 10 mg/kg significantly reduced immobility in 129/Sv mice ($F_{(3,36)} = 6.4$; $p < 0.01$), but the effect was less marked and no dose dependency was observed. Immobility time in DBA/2J ($F_{(5,49)} = 0.3$; $p > 0.05$) and BALB/c ($F_{(3,36)} = 1.2$; $p > 0.05$) mice was not affected by any dose of citalopram.

To evaluate the influence of cylinder size in the response to citalopram, we assessed the effect of the drug on immobility time using a 20-cm-wide cylinder. Basal immobility time in DBA/2J mice receiving vehicle was markedly reduced ($p < 0.001$, Student's t test) in the large cylinder (60.7 ± 11.3 s; $n = 7$) compared with the smaller cylinder (158.2 ± 10.4 s; $n = 12$), whereas no significant changes were observed in C57BL/6J mice (in the narrow and large cylinders, respectively, 165.0 ± 8.6 s, $n = 11$; and 156.0 ± 9.9 s, $n = 9$). With the 20 cm cylinder, 5 mg/kg citalopram significantly reduced immobility time in C57BL/6J mice

(98 ± 15.7 s; $n = 9$; $p = 0.006$ vs vehicle, Student's t test) but had no effect in DBA/2J mice (61.4 ± 15.9, $n = 6$; 67.8 ± 7.8, $n = 6$; and 58.6 ± 7.2, $n = 7$ s, in mice given 5, 10, and 20 mg/kg citalopram, respectively; $F_{(3,22)} = 0.1$; $p = 0.9$; one-way ANOVA).

Open-field activity

As reported in Table 2, significant differences in spontaneous locomotion were found between strains. 129/Sv mice were less active than C57BL/6J mice ($t_{(14)} = 13.3$; $p < 0.001$, Student's t test), whereas BALB/c were less active than DBA/2J mice ($t_{(15)} = 7.1$; $p < 0.001$, Student's t test). In general, DBA/2J mice were the most active, followed by C57BL/6J, BALB/c, and 129/Sv. However, the comparison of the four strains is only tentative because C57BL/6J and 129/Sv mice received only one injection of vehicle, whereas DBA/2J and BALB/c mice were given two. C57BL/6J and 129/Sv mice showed no change in the open-field activity after doses of citalopram that reduced immobility time in the FST, although a tendency to a reduction was observed in 129/Sv mice (C57BL/6J, $F_{(3,28)} = 1.3$, $p = 0.3$; 129/Sv, $F_{(3,28)} = 0.9$, $p = 0.5$).

Table 2 shows that TRP and citalopram singly or coadministered did not affect the locomotor activity of DBA/2J mice ($F_{TRP\ (1,28)} = 3.6$, $p > 0.05$; $F_{citalopram\ (1,28)} = 0.2$, $p > 0.05$; $F_{TRP\ \times\ citalopram\ (1,28)} = 0.72$, $p > 0.05$). In BALB/c mice, however, citalopram significantly enhanced open-field activity ($F_{citalopram\ (1,29)} = 16.5$; $p = 0.0003$). This effect was not modified by pretreatment with TRP ($F_{TRP\ \times\ citalopram\ (1,29)} = 2.1$; $p = 0.16$).

TRP had no effect on locomotor activity in BALB/c mice ($F_{TRP\ (1,29)} = 0.9$; $p = 0.3$).

Brain levels of citalopram

Mean citalopram brain concentrations rose with the dose in all strains. After 5 mg/kg citalopram, at the end of the behavioral test, levels were similar in all strains except 129/Sv mice, which had significantly higher levels than BALB/c mice (Table 3). Brain drug levels in 129/Sv mice given 2.5 mg/kg citalopram were significantly higher than in C57BL/6J and DBA/2J mice, but, in the latter strain, they were significantly lower than in BALB/c mice. Overall, ANOVA indicated a significant effect of strain ($F_{(3,67)} = 7.4$; $p = 0.0002$) and treatment ($F_{(1,67)} = 182.6$; $p < 0.0001$) but not strain × treatment interaction ($F_{(3,67)} = 2.7$; $p = 0.06$).

Citalopram concentrations in plasma were lower than in brain, with significant differences between strains. Plasma levels of citalopram in 129/Sv mice were higher than in BALB/c and C57BL/6J mice but similar to those in DBA/2J mice (data not shown). Mean plasma concentrations of N-demethylcitalopram increased with the citalopram dose in all strains. However, at 1.25 and 2.5 mg/kg, it was below the LOQ (data not shown). The metabolite was never detectable in the brain (< 0.08 μg/g).

Accumulation of 5-HTP and DOPA and concentrations of 5-HT

5-HT synthesis, measured by basal 5-HTP accumulation, in the whole brain of DBA/2J and BALB/c mice given vehicle was significantly lower than in other strains ($F_{(3,26)} = 18.3$; $p < 0.0001$). Brain 5-HTP content in DBA/2J and BALB/c mice was ~30% lower than in C57BL/6J and ~40% lower than in 129/Sv mice
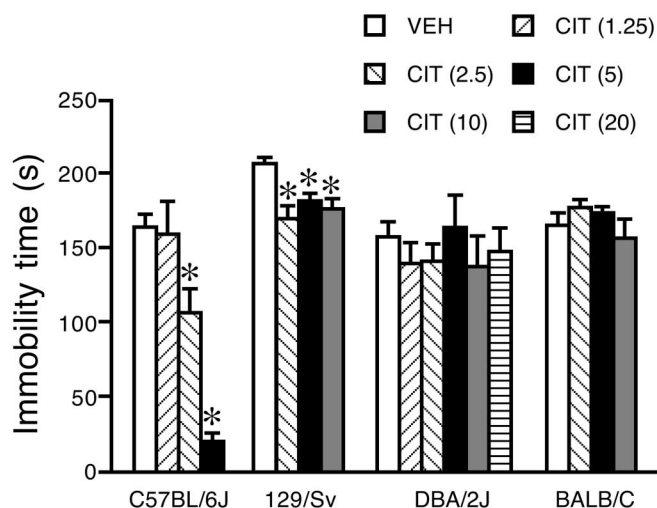
Figure 2. Strain differences in the effect of citalopram in the forced swimming test. Mice were given citalopram (CIT; doses expressed as milligrams per kilogram, in parentheses) 30 min before testing. Data are means ± SEM of 7–11 mice per group. *$p < 0.05$ versus respective vehicle (VEH), Tukey-Kramer's test.

Table 2. Open-field activity in C57BL/6J, 129/Sv, DBA/2J, and BALB/c mice given different doses of citalopram alone or in combination with TRP

Dose of citalopram (mg/kg)	C57BL/6J	129/Sv
Veh	150.9 ± 6.3 (n = 8)	27.1 ± 6.8 (n = 8)
1.25	143.8 ± 8.5 (n = 8)	ND
2.5	153.6 ± 10.4 (n = 8)	13.5 ± 6.2 (n = 8)
5.0	167.1 ± 7.9 (n = 8)	16.1 ± 10.6 (n = 8)
10.0	ND	11.3 ± 4.8 (n = 8)
Treatment	DBA/2J	BALB/c
Veh plus Veh	208.3 ± 12.3 (n = 8)	94.0 ± 10.3 (n = 9)
Veh plus Citalopram	232.6 ± 23.2 (n = 8)	160.3 ± 13.9* (n = 8)
TRP plus Veh	189.3 ± 14.6 (n = 8)	99.9 ± 4.7 (n = 8)
TRP plus Citalopram	182.9 ± 20.1 (n = 8)	131.5 ± 16.4 (n = 8)

Top, Effects of different doses of citalopram or vehicle (Veh) on open-field activity in C57BL/6J and 129/Sv mice. Bottom, Effects of TRP (300 mg/kg), citalopram (5 mg/kg), and vehicle alone or in combination on open-field activity in DBA/2J and BALB/c mice. Data are mean ± SEM. The number of mice is indicated in parentheses. *$p < 0.05$ versus Veh plus Veh (Tukey-Kramer's test). ND, Not determined.

(Fig. 3). Basal levels of 5-HTP in C57BL/6J and 129/Sv mice were not significantly different. Likewise, no differences were observed between DBA/2J and BALB/c mice.

The concentrations of 5-HT in the brain of untreated DBA/2J and BALB/c mice were, respectively, 426 ± 9 ng/g (n = 7) and 393 ± 13 ng/g (n = 6). These values were significantly lower (both $p < 0.05$, Tukey-Kramer's test) than in C57BL/6J (477 ± 11 ng/g; n = 7) and 129/Sv (493 ± 19 ng/g; n = 6) mice ($F_{(3,22)} = 12.1$; $p < 0.0001$).

Citalopram at 5 mg/kg inhibited brain 5-HT synthesis by 37% in C57BL/6J mice and by 42% in 129/Sv mice. Citalopram at 5 mg/kg had significantly less overall effect in DBA/2J and BALB/c mice than C57BL/6J and 129/Sv mice ($F_{\text{strain }(3,49)} = 22.9$, $p < 0.0001$; $F_{\text{treatment }(1,49)} = 74$, $p < 0.0001$; $F_{\text{strain} \times \text{treatment }(3,49)} = 5.5$, $p < 0.003$). The effect of 5 mg/kg citalopram was probably maximal because there was no additional reduction of 5-HTP accumulation at the dose of 20 mg/kg in C57BL/6J (−46%) and DBA/2J (−23%) mice. Citalopram at 20 mg/kg reduced 5-HTP accumulation in C57BL/6J mice more than in DBA/2J mice ($F_{\text{strain }(1,21)} = 14.1$, $p = 0.0011$; $F_{\text{treatment }(1,21)} = 63$, $p < 0.0001$; $F_{\text{strain} \times \text{treatment }(1,29)} = 15.2$, $p = 0.0008$) (Fig. 3). Basal striatal accumulation of 5-HTP in DBA/2J mice was 42% lower than in

C57BL/6J mice (data not shown). Citalopram reduced 5-HTP in the striatum of C57BL/6J mice by ~30% at both 5 and 20 mg/kg but had no significant effect in DBA/2J mice (data not shown).

No effects of strain or citalopram were found on the accumulation of whole brain DOPA (Fig. 3).

Effects of TRP and PCPA on 5-HTP accumulation and citalopram-induced immobility

In DBA/2J (Fig. 4b) and BALB/c (Fig. 4d) mice, 300 mg/kg TRP raised brain 5-HTP accumulation more than twofold but had no effect on DOPA accumulation. At 30 and 100 mg/kg, TRP had no effects on 5-HTP and DOPA accumulation.

As shown in Figure 4, a and c, a single dose of citalopram, which had no effect on immobility time in DBA/2J or BALB/c mice, significantly reduced immobility time in mice pretreated with 300 mg/kg TRP (DBA/2J, $F_{(1,36)} = 11.7$, $p < 0.01$; BALB/c, $F_{(1,36)} = 12.3$, $p < 0.01$), and TRP by itself did not change immobility time in both strains.

Doses of 100 mg $\cdot$ kg^{-1} $\cdot$ d^{-1} PCPA for 3 d reduced the accumulation of brain 5-HTP in C57BL/6J (Fig. 5b) and 129/Sv (Fig. 5d) mice by, respectively, 70 and 50% and prevented the reduction of immobility time by 5 mg/kg citalopram (C57BL/6J, $F_{(1,56)} = 6.7$, $p < 0.05$; 129/Sv, $F_{(1,36)} = 8.5$, $p < 0.01$) (Fig. 5a,c).

Brain levels of citalopram were not significantly affected by pretreatment with PCPA (C57BL/6J mice: vehicle plus citalopram, 1.5 ± 0.3 ng/g; PCPA plus citalopram, 1.6 ± 0.3 ng/g; 129/Sv mice: vehicle plus citalopram, 1.4 ± 0.2 ng/g; PCPA plus citalopram, 1.3 ± 0.1 ng/g). Likewise, TRP had no effect on brain levels of citalopram (DBA/2J mice: vehicle plus citalopram, 1.0 ± 0.1 ng/g; TRP plus citalopram, 1.0 ± 0.23 ng/g; BALB/c mice: vehicle plus citalopram, 1.2 ± 0.2 ng/g; TRP plus citalopram, 1.2 ± 0.1 ng/g).

Discussion

Citalopram reduced immobility time in the FST in C57BL/6J and 129/Sv but not in DBA/2J and BALB/c inbred mice even at four times higher doses. This strongly suggests that genetic differences play a role in this response. The different effects are not attributable to pharmacokinetic factors because brain citalopram levels were essentially similar in the four strains after the behavioral test. Slightly lower levels of citalopram in the brain of DBA/2J mice given 2.5 mg/kg citalopram or in BALB/c mice given 5 mg/kg are mostly corrected by increasing the dose and are unlikely to account for the lack of response of these strains in the FST. The main metabolite N-demethylcitalopram was undetectable in the brain of all strains, suggesting that it does not play a significant role in the effects of the parent drug.

Doses of citalopram reducing immobility time in C57BL/6J and 129/Sv mice had no effect on locomotor activity. Thus, the data on locomotion do not support the involvement of motor performance in the FST response to citalopram. Likewise, citalopram and paroxetine reduced the immobility time in NIH-Swiss mice at doses with no effect on locomotion (David et al., 2003). Citalopram had less anti-immobility effect in 129/Sv mice than in C57BL/6J mice, and no dose dependency was observed. This may be partly explained by the fact that this strain performs poorly in several tests involving locomotor activity and/or exploration, such as open field (present study) or Y maze and Barnes maze (Wahlsten et al., 2003). However, differences in locomotor activity can hardly explain the different effects of citalopram in the FST in the other strains.

The mouse strains used in the present study differ in several neurochemical parameters potentially involved in the antide-

Table 3. Brain levels of citalopram in C57BL/6J, 129/Sv, DBA/2J, and BALB/c mice given different doses of the drug intraperitoneally

Dose of citalopram (mg/kg)	C57BL/6J	129/Sv	DBA/2J	BALB/c
1.25	0.15 ± 0.03	ND	0.12 ± 0.04	ND
2.5	0.34 ± 0.09*	0.48 ± 0.16	0.25 ± 0.08[*,#]	0.40 ± 0.06
5.0	0.99 ± 0.24	1.33 ± 0.39	1.00 ± 0.10	0.87 ± 0.32*
10.0	ND	3.32 ± 0.62	2.95 ± 0.26	3.26 ± 0.87
20.0	ND	ND	5.75 ± 0.46	ND

At the end of the behavioral test, 37–40 min after citalopram injection, C57BL/6J ($n = 8$–10), 129/Sv ($n = 9$–10), DBA/2J ($n = 8$–10), and BALB/c ($n = 5$–10) mice (the same as in Fig. 2) were killed. Brain levels of citalopram were determined as described in Materials and Methods. Data are means ± SD. *$p <$ 0.05 versus 129/Sv; [#]$p <$ 0.05 versus BALB/c (Tukey-Kramer's test). ND, Not determined.
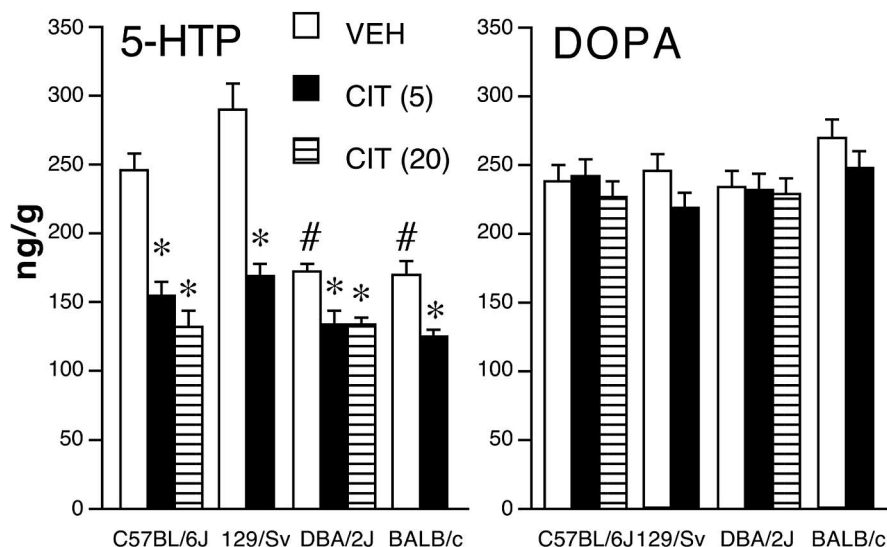

Figure 3. Effect of citalopram on whole-brain 5-HT and catecholamine synthesis as assessed by 5-HTP and DOPA accumulation after decarboxylase inhibition. Mice were given citalopram (CIT; doses expressed as milligrams per kilogram, shown in parentheses) 30 min before *m*-hydroxybenzylhydrazine and killed 30 min later. The citalopram-induced decrease of 5-HTP in DBA/2J (5–6 per group) and BALB/c (8 per group) mice was significantly less than in C57BL/6J (6–7 per group) and 129/Sv (8–9 per group) mice (for *F* values, see Results). Data are means ± SEM. *$p <$ 0.05 versus vehicle (VEH); [#]$p <$ 0.05 versus C57BL/6J and 129/Sv vehicle-treated mice (Tukey-Kramer's test).

pressant response. These include the content of brain noradrenaline (NA) (Kempf et al., 1974), the number of noradrenaline uptake sites in the locus ceruleus (Hwang et al., 1999), and the density of hippocampal glutamate, GABA, and 5-HT receptors (Zilles et al., 2000). Although we cannot exclude an effect of these differences, the results suggest that the failure of citalopram to reduce immobility in DBA/2J and BALB/c mice is mainly attributable to the genotype-dependent impairment of 5-HT synthesis.

This is supported by several findings. First, unresponsive strains (DBA/2J and BALB/c) are homozygous for the 1473G allele of TPH-2, whereas responsive strains (C57BL/6J and 129/Sv) are homozygous for the 1473C allele. This polymorphism, first reported by Zhang et al. (2004), was confirmed by the present study.

Second, in keeping with previous findings (Zhang et al., 2004), 5-HTP accumulation in the whole brain and striatum and 5-HT levels in the brain of DBA/2J and BALB/c mice were significantly lower than in the C57BL/6J and 129/Sv strains. Furthermore, citalopram inhibited brain 5-HT synthesis in C57BL/6J and 129/Sv mice but had significantly less effect in DBA/2J and BALB/c mice and no real effect in the striatum of DBA/2J mice. The fact that strain and citalopram had no effect on the accumulation of DOPA, the precursor of catecholamines, indicates the specificity of strain differences in 5-HTP accumulation and con-

firms that catecholamines are not involved in the antidepressant-like effect of citalopram (Cryan et al., 2004).

Third, enhancement of 5-HT synthesis by the 5-HT precursor TRP reinstated the anti-immobility effect of citalopram in DBA/2J and BALB/c mice, whereas the inhibition of 5-HT synthesis with PCPA prevented the antidepressant-like effect of citalopram in C57BL/6J and 129/Sv mice. This indicates that brain 5-HT synthesis plays a major role in the antidepressant-like effect of citalopram.

The effect of citalopram on immobility time in mice given TRP or PCPA cannot be accounted for by differences in drug disposition because brain concentrations immediately after the FST were not modified. Also, motor performance was not altered in DBA/2J mice given citalopram, TRP, or the combination. Although citalopram increased locomotor activity by itself, immobility was reduced in BALB/c mice given TRP plus citalopram without any change in locomotion.

Interestingly, PCPA and TRP did not change basal immobility time. Thus, stimulation, and particularly reduction of 5-HT synthesis, may be not sufficient to affect behavior in the FST. Previous studies reported no effects of PCPA or the serotonergic neurotoxin 5,7-dihydroxytryptamine on immobility time in mice and rats (Cervo and Samanin, 1991; Borsini, 1995; Redrobe et al., 1998; Page et al., 1999). Likewise, doses of TRP close to that we used had no effect on immobility time in NIH-Swiss mice, whereas at 50–125 mg/kg TRP reduced immobility (Hilakivi-Clarke et al., 1990; Wong and Ong, 2001).

The lack of response to citalopram in DBA/2J mice does not seem to be related to adaptive changes or abnormal 5-HT uptake because synaptosomal 5-HT uptake was similar in various brain regions of C57BL/6J and DBA/2J mice (Jazrawi and Horton, 1989). The failure of citalopram to reduce immobility in DBA/2J mice is consistent with another report that citalopram and paroxetine did not affect immobility time in this strain (David et al., 2003). However, Lucki et al. (2001) showed that fluoxetine reduced immobility time in DBA/2J and BALB/c mice, had no effect in C57BL/6J, and increased immobility in 129/SvemJ mice. Although this apparently argues against a role for allelic differences in TPH-2 in the effect of SSRIs, the genotype–environment interaction may cause differences in behavioral phenotype across laboratories (Crabbe et al., 1999; Wahlsten et al., 2003; Kafkafi et al., 2005). Differences in the SSRIs, substrain, and procedures used may also contribute.

In particular, differences in the effects of SSRIs in the FST may reflect differences in selectivity among SSRIs. Fluoxetine, at doses increasing extracellular 5-HT, also raised extracellular dopamine (DA) and NA in the rat medial prefrontal cortex (Jordan et al., 1994; Tanda et al., 1994; Pozzi et al., 1999), and its effect on DA was not modified by neurotoxic lesion of 5-HT neurons with 5,7-dihydroxytryptamine, which completely prevented the rise of
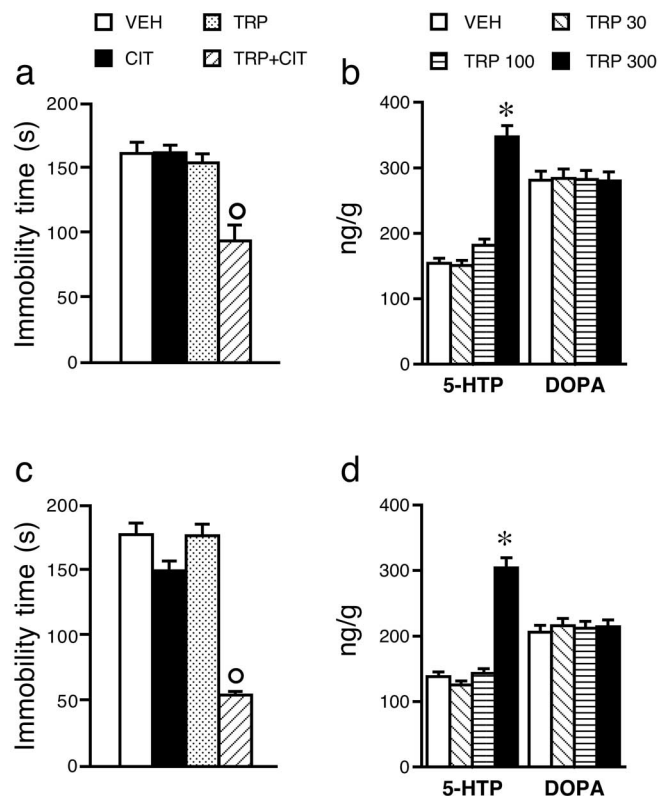
Figure 4. Effect of TRP and citalopram (CIT) coadministered on immobility time and accumulation of brain 5-HTP and DOPA in DBA/2J (*a, b*) and BALB/c (*c, d*) mice. Mice received vehicle (VEH) or 300 mg/kg intraperitoneal tryptophan 30 min before saline or 5 mg/kg citalopram (CIT) and were tested 30 min after citalopram (*a, c*; $n = 10$ per group). The effect of 30–300 mg/kg tryptophan on 5-HTP and DOPA accumulation in DBA/2J (*b*) and BALB/c (*d*) mice was assessed in separate groups of six mice. Data are means ± SEM. *$p < 0.05$ versus respective vehicle; °$p < 0.05$ versus citalopram (Tukey-Kramer's test).

Figure 5. Effect of PCPA on citalopram-induced reduction of immobility time and accumulation of brain 5-HTP and DOPA in C57BL/6J (*a, b*) and 129/Sv (*c, d*) mice. C57BL/6J ($n = 15$ per group) and 129/Sv ($n = 10$ per group) mice were given vehicle (VEH) or PCPA (100 mg/kg orally for 3 consecutive days). Twenty-four hours after the last dose, they were given saline or 5 mg/kg citalopram (CIT) and tested 30 min later. Separate groups of C57BL/6J (*b*) and 129/Sv (*d*) mice were given PCPA or vehicle ($n = 5$–6 per group), and brain 5-HTP and DOPA accumulation was measured 24 h after the last dose. Data are means ± SEM. *$p < 0.05$ versus respective vehicle; °$p < 0.05$ versus citalopram (Tukey-Kramer's test).

extracellular 5-HT (Pozzi et al., 1999). Although 5-HT depletion prevented the anti-immobility effect of fluoxetine in the FST in rats and mice (Page et al., 1999; Rodrigues et al., 2002; Gavioli et al., 2004), it had much less effect (like that of paroxetine and sertraline) in dopamine β-hydroxylase (Dbh) knock-out mice (Cryan et al., 2004), whereas the anti-immobility effect of sertraline in rats was not affected by 5-HT depletion (Cervo et al., 1991). Unfortunately, Lucki et al. (2001) did not assess whether the anti-immobility effect of fluoxetine in DBA/2J mice depends on endogenous 5-HT.

Citalopram is reported to inhibit NA reuptake in NA transporter knock-out mice (Vizi et al., 2004), but it has no effect on extracellular NA in animals with a functional NA transporter, unless very high doses are used (Pozzi et al., 1999). Thus, the antidepressant-like effect of citalopram was completely prevented by the 5-HT synthesis inhibitor PCPA (present study) but was unchanged in mice with deletion of the NA transporter (Vizi et al., 2004) or the Dbh genes (Cryan et al., 2004).

It has been suggested that, when the diameter of the cylinder used to assess immobility is 20 cm or bigger, there may be fewer false-positive errors in the mice FST (Sunal et al., 1994; Petit-Demouliere et al., 2005). In line with previous findings (David et al., 2003), we observed no differences in basal immobility time between C57BL/6J, DBA/2J, and BALB/c mice using the 10 cm cylinder, whereas 129/Sv mice were more immobile than other strains. However, using the 20 cm cylinder, basal immobility time was strongly reduced in DBA/2J but not in C57BL/6J mice. Con-
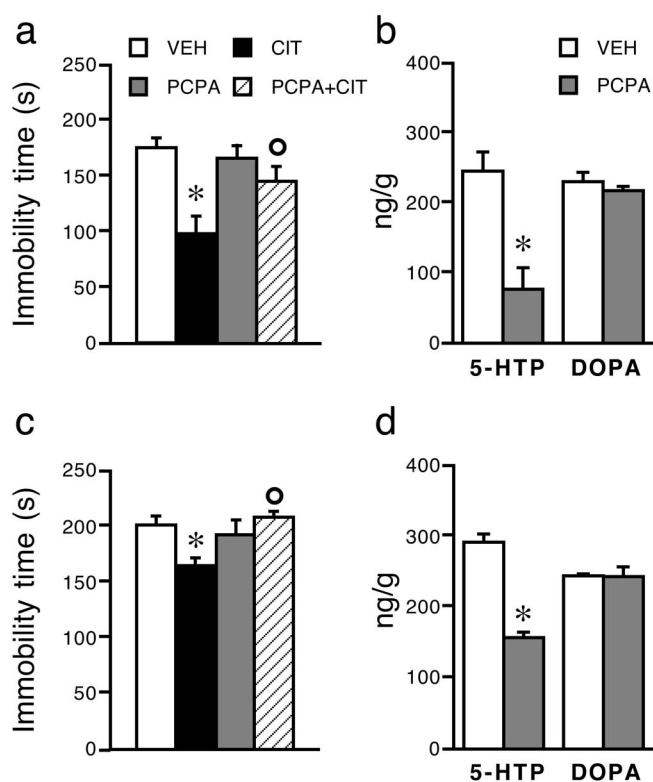
sistently, DBA/2J mice were less immobile than C57BL/6J mice in a 20 cm cylinder (Lucki et al., 2001). Despite the marked changes in basal immobility, the different responses of C57BL/6J and DBA/2J mice to citalopram were not affected by the size of the cylinder. Thus, the effect of established antidepressant drugs in the FST did not depend on the cylinder diameter (Sunal et al., 1994), which is therefore unlikely to account for the differences between the present findings and those of Lucki et al. (2001). It remains to be clarified whether other procedural differences contributed.

Active behavior in the FST can be distinguished into climbing and swimming (Cryan et al., 2002a). Mice with shorter immobility time in response to citalopram alone (C57BL/6J and 129/Sv) or with TRP (DBA/2J and BALB/c) increased their active swimming, with no significant effect on climbing (A. Canetta and L. Cervo, unpublished observations). These different behavioral profiles are consistent with previous reports that antidepressants blocking 5-HT reuptake preferentially increase swimming in rats (Detke et al., 1995; Reneric and Lucki, 1998) and mice (Alcaro et al., 2002).

SSRI-induced inhibition of 5-HT synthesis depends on the ability of the drugs to enhance endogenous 5-HT tone on 5-HT receptors, which in turn inhibits 5-HT synthesis (Stenfors et al., 2001). Because the inhibition of 5-HT synthesis by citalopram is reversed by 5-HT$_{1A}$ and 5-HT$_{1B/1D}$ receptor antagonists (Barton and Hutson, 1999), it has been suggested that autoreceptor activation plays a major role. However, other studies failed to con-

firm this and suggested that some other 5-HT receptor subtype contributes (Moret and Briley, 1997; Stenfors et al., 2001). Interestingly, TRP and the activation of TPH by Ca/calmodulin kinase II and protein kinase A enhanced 5-HT$_{1A}$ receptor-mediated inhibition of dorsal raphe 5-HT neurons (Liu et al., 2005). Thus, autoreceptor-mediated inhibition of brain 5-HT synthesis is quite likely impaired in DBA/2J and BALB/c mice.

The present results suggest that strategies to stimulate 5-HT synthesis might enhance the antidepressant effect of SSRIs in depressed patients carrying the A/A and G/A alleles of TPH-2, who, according to the only available study, may amount to 10% (Zhang et al., 2005). Clinical studies have shown that the therapeutic effect of SSRIs in drug-remitted depressed patients was reversed by the 5-HT synthesis inhibitor PCPA and by TRP depletion (Shopsin et al., 1976; Delgado et al., 1990). However, the combination of TRP with fluoxetine, zimelidine, clomipramine, and amitryptyline has only occasionally achieved any potentiation or acceleration of the clinical effect, and we do not know how TRP augmentation affect patients not responding to SSRIs (Young, 1991; Young and Leyton, 2002). Thus, it would be important to establish whether this strategy improves the therapeutic outcome in this subgroup, particularly patients carrying the A/A and G/A alleles of TPH-2.

In conclusion, the results provide functional evidence that the genotype-dependent regulation of 5-HT synthesis is an important factor in the antidepressant-like action of citalopram. Interstrain comparisons are important for a clearer understanding of the mechanisms underlying the response to SSRIs.

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