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Aging impairs the antidepressant-like response to citalopram in male rats

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ABSTRACT

It has been suggested that old depressed patients require longer antidepressant treatments than their young counterparts. The objective of this study was to establish if aging impairs the response to an antidepressant by using an animal model. For this purpose, young and middle-aged male Wistar rats (of around 4 and 14 months, respectively) were exposed to a chronic mild stress schedule for 3 weeks. After this period, the animals that developed anhedonia, reflected as a reduction in sucrose solution (1%) intake, were treated with citalopram (10 mg/kg/day) during 21 days while still maintained under the chronic mild stress schedule. Non-stressed animals were included as controls. In young rats citalopram reversed the reduction in sucrose consumption induced by chronic mild stress after one week of treatment, while in middle-aged animals a similar reversion occurred after three weeks. Citalopram did not importantly modify simple water intake in stressed animals or sucrose consumption in non-stressed rats of both ages. The results imply that young rats have a lower latency of onset to the antidepressant-like effect of citalopram than middle-aged animals. The lower sensitivity of middle-aged animals to citalopram could be underlied by their lower levels of gonadal hormones.

1. Introduction

Depression is a mood disorder characterized by anhedonia (incapacity to experience pleasure), hopelessness, sadness, guilt feelings and suicide thoughts (American Psychiatric Association, 2000). Currently, the incidence of depression is increasing worldwide and it is estimated that by 2020 it will be the second cause of incapacity and mortality (Murray and López, 1997). This disorder is widespread among old people and this proportion is increasing each year. Depression is particularly prevailing (10% to 25%) in people with chronic illnesses such as ischemic heart disease, stroke, cancer, chronic lung disease, arthritis, Alzheimer's and Parkinson's diseases (Reynolds and Kupfer, 1999).

It has been reported that the onset of action of antidepressants is longer in aged than in young patients (Reynolds and Kupfer, 1999; Reynolds et al., 1996), suggesting that factors associated with aging impair the response to these compounds. In males, such factors may include changes in the hypothalamus–pituitary–adrenal (HPA) and –gonadal (HPG) axes, which are associated with alterations in plasmatic levels of ACTH, glucocorticoids and testosterone, respectively (Lamberts et al., 1997). Although the age-dependent glucocorticoid increase is suggested to be one of the key factors to explain the blunted antidepressant response in aged patients (Kakiuchi et al., 2001; Yau et al., 1999), it is possible that the age-related decrease in testosterone also participate in this process (Martínez-Mota and Fernández-Guasti, 2004; Martínez-Mota et al., 2008; Pope et al., 2003; Seidman and Rabkin, 1998).

In line with clinical studies (Lebowitz et al., 1997), we recently found that aging increases the vulnerability of male rats to develop experimental depression when subjected to the chronic mild stress paradigm (Herrera-Pérez et al., 2008). This paradigm has been developed in laboratory animals to model the relatively minor and unanticipated irritations of human everyday-life (Willner et al., 1987). It is a highly validated animal model of depression, in which rodents are exposed to several low grade stressors (such as stroboscopic light, cage tilt, soiled cage, and white noise) during several weeks to induce anhedonia, a core symptom of human depression (Willner, 1997). The anhedonic state is inferred by a decrease in the consumption of a palatable sucrose solution and it is specifically reversed by chronic antidepressant treatments (Montgomery et al., 2001; Willner et al., 1987).

The aim of this study was to compare the response to citalopram between middle-aged (around 14 months) and young (around 4 months) male rats exposed to chronic mild stress. These two age groups were chosen since we previously demonstrated a reduction of about 75% in the levels of gonadal hormones in middle-aged animals compared to young rats (Herrera-Pérez et al., 2008). Citalopram was selected given that it exhibits: 1) a high selectivity for the serotonin transporter (SERT), an action that importantly reduces collateral effects; 2) a low risk of pharmacological interactions, a relevant

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feature since aged patients are frequently poly-medicated (Solai et al., 2001; Spina and Scordo, 2002), and 3) a linear kinetic along its therapeutic range, which allows an adequate dose control (Overo, 1982). All these characteristics make this selective serotonin reuptake inhibitor (SSRI) a suitable treatment for depression in the elderly (Keller, 2000).

2. Materials and methods

2.1. Animals

Young and middle-aged (3–5 and 12–15 months, respectively) male Wistar rats were obtained from the Instituto Nacional de Psiquiatría "Ramón de la Fuente Muñiz". Animals were individually housed and maintained on a 12 h inverted dark–light cycle (lights on at 22:00 h), under controlled temperature and humidity. The rats had free access to water and food, except for the periods required by the chronic mild stress procedure. Animal management was done according to the general principles of laboratory animal care (NIH publication 85-23, 1985). All experimental procedures were performed in accordance with the Mexican official norm for animal care and handling (NOM-062-ZOO-1999) and approved by the Ethical Committee of the "CINVESTAV-IPN" and Instituto Nacional de Psiquiatría "Ramón de la Fuente Muñiz". All efforts were made to minimize the number of animals used and their suffering.

2.2. Chronic mild stress model

2.2.1. Sucrose consumption

Rats were allowed to adapt to the taste of a palatable sucrose solution (1%) for two consecutive weeks. During this period, a bottle containing sucrose solution was presented to the rats daily for 1 h, at the beginning of the dark phase. The basal sucrose consumption was determined. For this purpose, the rats were water and food deprived for 20 h and thereafter presented with two bottles during 1 h: one containing sucrose solution (1%) and the other tap water. Fluid (sucrose solution or tap water) consumption was calculated by weighing the bottles before and after their exposure to the animals.

2.2.2. Experimental design

Young and middle-aged male rats were divided into two groups (control and stressed), matched for similar basal sucrose consumption. In the control group, the males were individually housed during six weeks without stress, but under comparable handling and storage conditions than the experimental group. The stress group was exposed, during six weeks, to several stressors: white noise (~90 dB), group housing (2–3 animal per cage), continuous light, soiled cage (250 ml of water spilled into the cage bedding), stroboscopic light (300 flashes/min), 45° cage tilt along the vertical axis and water deprivation; the schedule of stressors is shown in Table 1.

Sucrose solution and tap water consumption was determined weekly in both groups, after a 20 h period of water and food deprivation. This test consisted of an hour exposure to two bottles: one with sucrose solution and the other with tap water. The anhedonic state generated by the chronic mild stress schedule was reflected as a reduction in sucrose consumption of at least 2 g (Herrera-Pérez et al., 2008).

In line with previous findings (Herrera-Pérez et al., 2008), 20% of young and 73% of middle-aged rats developed anhedonia after three weeks of chronic mild stress. These anhedonic rats were selected and then divided into two groups, one that received citalopram (10 mg/kg/day) (*anhedonic stressed citalopram*; 6 young and 7 middle-aged rats) and the other saline solution (*anhedonic stressed vehicle*; 5 young and 7 middle-aged rats) during 21 days, both groups were continuously exposed to the mild stressors for the three weeks of treatment. The response to the antidepressant was reflected as an increase in sucrose consumption. Three weeks after the beginning of the experiment, control non-stressed rats were allocated into two groups and administered with saline solution (*control vehicle*; 7 young and 8 middle-aged rats) or citalopram (*control citalopram*; 7 young and 8 middle-aged) following the schedule previously described. All sucrose and tap water intakes for the control and stressed groups were determined at 10:00 h. Non-stressed rats were treated with citalopram to control for putative unspecific effects of this drug; measurements of tap water intake were done for the same reason.

2.3. Drugs

The racemic form of citalopram hydrochloride (kindly provided by *Laboratorio Médico Químico Biológico, S.A. de C.V.* México City, México) was dissolved in saline solution (10 mg/2 ml) and administered intraperitoneally (i.p.) at a dose of 10 mg/kg/day during three weeks. Saline solution was administered i.p. at a dose of 2 ml/kg/day. This dose of citalopram was selected since previous data revealed its effectiveness in this animal model (Montgomery et al., 2001).

2.4. Statistics

The data of water or sucrose solution intake were analyzed using three-way analysis of variance (ANOVA) including the factors age, treatment and weeks of treatment, followed by two-way repeated measures analyses of variance (RM ANOVA) and Tukey as the *post hoc* test. The Student's *t* test was used to compare two specific groups. A value of $P<0.05$ was considered as statistically significant.

3. Results

The sucrose intake of young and middle-aged anhedonic stressed rats showed statistically significant differences. Thus, the three-way ANOVA indicated a significant effect of age ($F_{1,84}=7.325$, $P=0.008$), treatment ($F_{1,84}=24.990$, $P<0.001$) and age and treatment interaction ($F_{1,84}=5.995$, $P=0.016$); but not for weeks of treatment, although the value was nearly significant ($F_{3,84}=2.65$, $P=0.054$).

In non-stressed rats, the three-way ANOVA indicated that sucrose solution consumption was not significantly influenced by age ($F_{1,104}=3.366$, $P=0.069$), treatment ($F_{1,104}=0.773$, $P=0.381$), weeks of treatment ($F_{3,104}=0.359$, $P=0.783$) nor by the interaction between these factors. This result indicates that citalopram did not affect the consumption of sucrose solution by itself.

Table 1
Chronic mild stress schedule.

Time (h)	First WED	THU	FRI	SAT	SUN	MON	TUE	Every subsequent WED
7:00–8:00			SC/CL	WD			GH/CL	FD/WD
8:00–9:00			SC/CL	WD			CT/CL	FD/WD
9:00–10:00			CL	WD			CT/CL	FD/WD
10:00–11:00			SL	WN			CT/CL	TEST
11:00–12:00		GH	SL	WN			CT/CL	
12:00–13:00		GH	SL	WN		SL	CT	
13:00–14:00	WN	GH	SL	WN		SL	CT	WN
14:00–15:00	WN	GH	SL			SL	FD/WD	WN
15:00–16:00	WN	GH/CL				SL	FD/WD	WN
16:00–17:00		SC/CL	WD			SL	FD/WD	
17:00–18:00		SC/CL	WD			GH/CL	FD/WD	
18:00–19:00		SC/CL	WD			GH/CL	FD/WD	
19:00–20:00		SC/CL	WD			GH/CL	FD/WD	
20:00–21:00		SC/CL	WD			GH/CL	FD/WD	
21:00–22:00		SC/CL	WD			GH/CL	FD/WD	
22:00–7:00		SC/CL	WD			GH/CL	FD/WD	

WN: White noise, GH: Grouped housing (2–3 rats per cage), CL: Continuous light, SC: Soiled cage, SL: Stroboscopic light, WD: Water deprivation, CT: Cage tilt (45°), FD: Food deprivation.

Fig. 1A shows that young anhedonic stressed rats treated with citalopram began to increase their sucrose consumption from the first week of treatment onwards, reaching levels of consumption similar to those of control animals (control vehicle) after two weeks of treatment. In contrast, the anhedonic stressed rats treated with vehicle (anhedonic stressed vehicle) showed no change in their low level of sucrose consumption. The two-way RM ANOVA indicated that the sucrose consumption of anhedonic stressed young rats treated with citalopram or vehicle was affected by treatment ($F_{1,9}=9.21$, $P=0.014$) and weeks of treatment ($F_{3,27}=3.19$, $P=0.040$). In the stressed group of anhedonic young rats treated with citalopram, *post hoc* comparisons indicated differences in sucrose consumption at weeks 2 and 3 compared with their consumption at week 0 (immediately before the beginning of the treatment). Significant differences in sucrose intake, at weeks 1, 2 and 3 of the treatment were also found between vehicle- and citalopram-anhedonic stressed young rats.

Tap water consumption of young anhedonic stressed rats is shown in Fig. 1B, the two-way RM ANOVA indicated only a statistical significant effect of time ($F_{3,27}=6.97$, $P=0.001$) without significance for treatment ($F_{1,9}=1.37$, $P=0.272$) or in the interaction between both factors ($F_{3,27}=1.513$, $P=0.234$). *Post hoc* analysis indicated that, in the young anhedonic stressed males treated with vehicle, tap water consumption was higher in weeks 1 and 2 when compared to week 0. Treatment with citalopram did not modify tap water intake suggesting that the increased sucrose intake of the anhedonic citalopram group (Fig. 1A) was a specific antidepressant-like effect of this SSRI.

Fig. 2A shows that middle-aged anhedonic stressed rats treated with citalopram increased their sucrose intake after 2 weeks and it reached the levels shown by control non-stressed animals until the third week. This behavioural response to citalopram contrasts with that shown by young anhedonic stressed animals. The stressed anhedonic middle-aged rats treated with vehicle did not change their sucrose consumption due to treatment. The two-way RM ANOVA indicated that sucrose consumption was affected by weeks of treatment ($F_{3,36}=8.375$, $P=0.003$); the Tukey test showed that sucrose intake after 3 weeks of citalopram treatment was higher than that at week 0 and than that of the anhedonic vehicle-treated group at the third week of treatment.

Tap water consumption of stressed anhedonic middle-aged rats is shown in Fig. 2B. The two-way RM ANOVA indicated that water intake was affected by weeks of treatment ($F_{3,36}=3.492$, $P=0.025$), but not by treatment ($F_{1,12}=0.0910$, $P=0.359$) nor by their interaction ($F_{3,36}=1.027$, $P=0.392$). *Post hoc* analysis indicated that tap water intake was reduced after three weeks of vehicle administration. This observation suggests that citalopram produced a specific antidepressant-like effect on the anhedonic state of middle-aged animals.

When comparing the sucrose intake of young and middle-aged stressed anhedonic rats treated with citalopram it is clear that before the initiation of treatment (week 0) both groups had similar levels of sucrose consumption ($t=0.805$; $P=$NS). However, one and two weeks after citalopram, the sucrose intake of young rats was significantly higher than that of middle-aged rats ($t=2.665$; $P=0.022$ and $t=2.251$; $P=0.046$, for weeks 1 and 2, respectively) (panels A, Figs. 1 and 2). After three weeks of treatment, sucrose consumption in both age groups was similar ($t=0.883$, $P=$NS). These data, taken together, indicate that middle-aged anhedonic rats respond later than young ones to the antidepressant-like effect of citalopram.

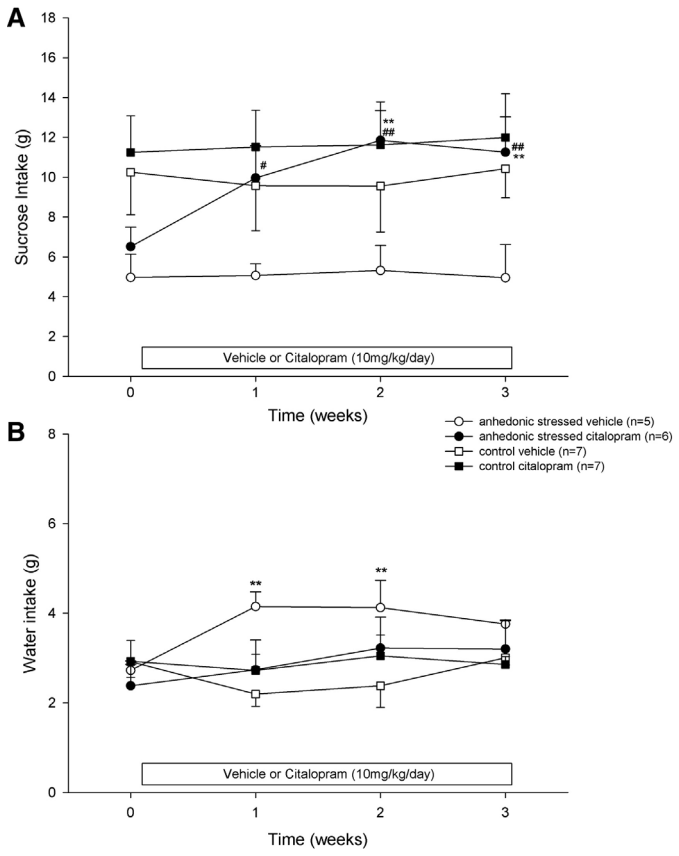

Fig. 1. Sucrose (A) and tap water (B) intake of young anhedonic stressed rats (circles) and controls (squares), treated with citalopram (full symbols) or vehicle (empty symbols). Data are expressed as means ± SEM. Tukey's test: $^{**}P<0.01$ *vs.* week 0; $^{\#}P<0.05$, $^{\#\#}P<0.01$ *vs.* anhedonic vehicle group.

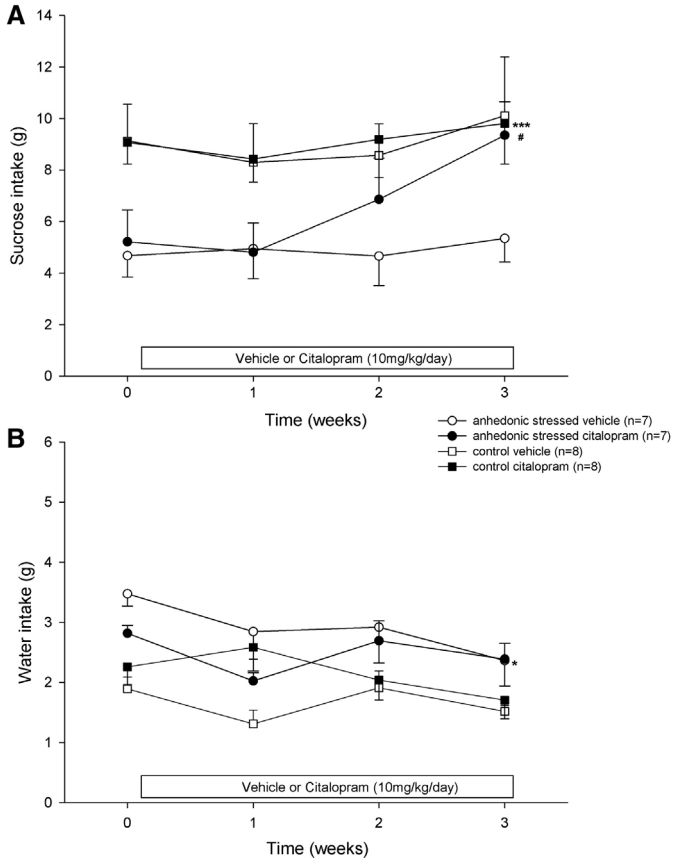

Fig. 2. Sucrose (A) and tap water (B) intake of middle-aged anhedonic stressed rats (circles) and controls (squares), treated with citalopram (full symbols) or vehicle (empty symbols). Data are expressed as means ± SEM. Tukey's test: $^{*}P<0.05$, $^{***}P<0.001$ *vs.* week 0; $^{\#}P<0.05$ *vs.* anhedonic vehicle group.

4. Discussion

In this study we found that young anhedonic animals exposed to chronic mild stress showed an antidepressant-like response after one week of treatment, while middle-aged rats responded after two weeks. The temporal course of the antidepressant-like response in young animals confirms the results obtained by Montgomery et al. (2001). In line with these results, David et al. (2001) found that imipramine (a tryciclic antidepressant), maprotiline (a noradrenaline reuptake inhibitor), fluvoxamine and sertraline (serotonin reuptake inhibitors) were more effective as antidepressants in 4-week-old mice than in 40-week-old mice, as evaluated in the forced swimming test. Furthermore, clinical reports showed that old depressed patients had a deficient response to antidepressant treatments, including desipramine (Nelson et al., 1995) and nortripiline (Little et al., 1998a).

The antidepressant effect of SSRIs may require the blockade of a certain number of serotonin transporters (SERT) (Kakiuchi et al., 2001). Accordingly, reduced expression of SERT would explain the impaired response to SSRIs. In support, Yu et al. (2002) reported that depressed patients who have reduced SERT expression — because they possess the short form of the SERT gene (Lesch et al., 1996; Little et al., 1998b) — also showed a reduced response to fluoxetine when compared to individuals who have the long form of the gene. A study done by Kakiuchi et al. (2001) in male *rhesus* monkeys showed that aging was associated to reduced SERT expression in brain areas such as the frontal cortex and hippocampus, which are involved in the response to antidepressant drugs (Santarelli et al., 2003). Taken together, these evidences support the hypothesis that the impaired response to citalopram observed in middle-aged anhedonic animals is, at least partially, explained by an age-related decrease in brain SERT expression (Kakiuchi et al., 2001), however, this idea needs to be tested.

The present study and a previous report (Herrera-Pérez et al., 2008) revealed that a much higher percentage of middle-aged rats, as compared to young, developed anhedonia after chronic mild stress. This age difference may be related to the reduction of about 75% in the levels of gonadal hormones in middle-aged rats (Gray, 1978; Herrera-Pérez et al., 2008). Gonadal hormones are known to influence the affective- and mental-state through a modulatory role on the serotoninergic transmission, among other systems. In line, a study done by Mc Queen et al. (1999) showed that castration of young (3 month old) male rats reduced SERT expression in the dorsal raphe, whereas testosterone or estradiol restitution reversed the effect. This observation suggests that a decrease in the levels of testosterone and estradiol is paralleled by a reduction in SERT and thereby an impaired response to SSRIs. In agreement, we recently showed that castration of young (4 months) male rats prevented the antidepressant-like effect of fluoxetine in the forced swimming test (Martínez-Mota and Fernández-Guasti, 2004), an action that was reversed by estradiol (Martínez-Mota et al., 2008). Although the blocking actions of gonadal steroid withdrawal (on the antidepressant-like effect of fluoxetine and on SERT expression) have been demonstrated in castrated young males, these data support the idea that the reduction of testosterone and estradiol observed in middle-aged animals (Herrera-Pérez et al., 2008) could underlie the delayed response to citalopram (present results). Additionally, estradiol has been shown to shorten the latency of onset of fluoxetine in ovariectomised young rats (Estrada-Camarena et al., 2008). Finally, the modulating action of gonadal hormones on the effects of SSRIs has also been observed in the clinic. Thus, it has been shown that testosterone supplementation improves refractory depression in men (Pope et al., 2003) and recovers the antidepressant effect of SSRIs in hypogonadal men with major depression resistant to antidepressants (Seidman and Rabkin, 1998).

Some evidences show that the antidepressant-like effects of some drugs require hippocampal neurogenesis. Santarelli et al. (2003) found that blocking cell proliferation in hippocampus, through X-ray radiation, deteriorates the antidepressant-like effect of some drugs in rodents. In agreement with this study, Jayatissa et al. (2006), using the chronic mild stress model, found two populations of anhedonic rats: one that responded to the treatment with escitalopram (an enantiomer of citalopram) and another that did not. The antidepressant-like response was positively correlated with the number of cells that proliferated in the hippocampus. These results suggest that the recovery from the anhedonic state requires hippocampal neurogenesis. In aged rats, deteriorated neurogenesis (Cameron and McKay, 1999) could be asociated to the delayed response to citalopram observed in the present study.

Alternatively, the age difference in the response to the antidepressant-like effect of citalopram could be explained by pharmacokinetic alterations. It is known that aging is associated with a reduction in enzymatic activity (citochrome P-450) of the rat liver (Fujita, 1991) and that citalopram is metabolized through this pathway (Rochat et al., 1998). However, middle-aged animals would have higher plasmatic levels of citalopram than young ones after receiving a similar dose. Thus, this idea does not explain a delayed response in middle-aged rats.

Citalopram treatment did not modify sucrose consumption in control (non-stressed) young or middle-aged rats, suggesting that this treatment has a specific antidepressant-like effect in anhedonic rats. This observation also agrees with clinical data showing that citalopram selectively modifies the hedonic state of depressed patients without producing affective changes in healthy volunteers (Willner, 1997). One of the most common collateral effects of antidepressants is thirst (Stahl, 2000). Since chronic mild stress evaluates the antidepressant-like effect of citalopram as an increased intake of sucrose solution, it is possible to propose that such increased ingestion is the consequence of thirst. In order to evaluate such an effect, tap water intake was measured all over the experiment. Interestingly, water intake increased in young animals treated with vehicle, while it decreased in similarly treated middle-aged males. The sucrose solution intake for each group was constant throughout the course of the experiment. In contrast, anhedonic animals treated with citalopram did not change their tap water intake during the experiment; whereas their sucrose consumption increased weekly. Evidently, the variations in tap water intake did not correlate with the variations in sucrose consumption. These observations, together with the fact that citalopram treatment did not alter tap water intake in non-stressed rats, validate the specificity of the antidepressant-like effect of this drug, discarding unspecific effects on fluid intake.

5. Conclusion

This study demonstrates that middle-aged rats show a delayed response to the antidepressant-like effects of citalopram. Such delay may be related to an age-dependent reduction in gonadal hormones. Additionally, the present results suggest that the chronic mild stress model is a useful tool to determine the putative role of different neuroendocrine conditions in the age-associated impaired response to antidepressants.

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