Antidepressant-like effect of low dose of scopolamine in the H/Rouen genetic mouse model of depression

Running title: Scopolamine in H/Rouen mice.

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Abstract

Rodent models of depression are useful for the investigation of cellular and neuronal mechanisms of antidepressant drugs and for the discovery of potential new targets. In this study, we examined the antidepressant-like effect of scopolamine, a non-selective muscarinic antagonist, in a genetic mouse model of depression obtained through a selective breeding strategy and called H/Rouen. In this model, we observed that scopolamine was active both in males and females at a lower dose (0.03 mg/kg) in the tail suspension test, 30 min following its administration, than observed in CD-1 mice. In addition, we showed this antidepressant-like effect was partly inhibited by an injection of 10 mg/kg of the AMPA receptor antagonist NBQX in both males and females, suggesting the anti-depressant like effect of scopolamine was mainly driven by AMPA receptors in the H/Rouen mouse line. Altogether, our results showed the high sensitivity of the H/Rouen mouse model of depression to study the antidepressant-like effects of pharmacological compounds.

Keywords: scopolamine; NBQX; tail suspension test; mouse model; depression, male and female

Introduction

Most of the antidepressants available on the market are based on the monoamine hypothesis of depression and aim at increasing monoamine neurotransmission in patients with a major depressive disorder [1]. However, these drugs are not efficient for all patients and one-third of them still manifest symptoms after 4 different medication courses [2]. Evidence has been obtained for a rapid effect of the non-selective muscarinic receptors antagonist scopolamine in depressed patients [for reviews see 3, 4, 5]. In preclinical studies performed in rodents, high doses of scopolamine have revealed a good efficiency to decrease the immobility time in the tail suspension test and the forced swim test [6, 7], two tests particularly relevant to study antidepressant-like effects [8, 9]. One major hypothesis concerning the rapid effects of scopolamine concerns the involvement of a glutamatergic burst [10].

Using CD-1 outbred mice selective breeding-based on behavioral reaction after an acute mild stress, we previously generated a mouse model, called H/Rouen [11]. This model was obtained after mating animals with the lowest mobility in the tail suspension test (TST). In addition to the low mobility observed in the TST, these animals manifested depressive-like behaviors, anhedonia-like behaviors, anxiety-like behaviors, impairment in sleep patterns, and a high basal corticosterone level [11-13], which were reversed by chronic treatment with serotonin reuptake inhibitors [11]. Here we investigated the effects of scopolamine, a rapid-acting antidepressant on this mouse model. We first demonstrated that scopolamine is active at very low doses in the tail suspension test (TST) following its administration to both male and female H/Rouen mice. Next, we showed that the antidepressant-like effect of scopolamine was significantly reduced by a pretreatment with the 2,3-dihydroxy-6-nitro-7-sulfamoyl-

benzo[f]quinoxaline (NBQX), an α-amino-3-hydroxy-5-méthylisoazol-4-propionate acid (AMPA) receptor antagonist.

Experimental procedures

Animals

H/Rouen mice have been bred in the University Lyon 1 animal facility and selected for their low mobility in the TST as previously described [11]. They were kept on a 7 a.m.-7 p.m. light cycle with food and water ad libitum. Testing was performed between 9 a.m. and 5 p.m.. All experiments were conducted in accordance with the regulations of the local ethical committee (Lyon 1 University CE2A- UCBL BH 2009-03) and the European Community Council Directive of November the 24th, 1986 (86/609/EEC).

Drugs

Scopolamine hydrochloride (Sigma-Aldrich, St Louis, MO, U.S.A.) and NBQX disodium salt hydrate (Sigma-Aldrich) were dissolved daily in vehicle. Doses always refer to the free bases.

Tail Suspension Test

The TST was performed with a computerized device. Mice were suspended by the tail with adhesive tape to a hook connected to a strain gauge. This gauge transmitted movements to a computer calculating total duration of immobility during a 6-min test. Mice that climbed up their tail during the test session were excluded from the study.

Drugs treatments

Acute treatments were performed on adult mice (10 to 14-week-old). Drug administration were performed intraperitoneally. Mice received either an acute injection of scopolamine or of a vehicle. All mice were tested with the TST 30 minutes after injection. When tested, NBQX was administered 45 min before the test, i.e. 15 minutes before the injection of scopolamine or its vehicle.

Statistical Analysis

Results were expressed as median ± interquartile range. Statistical analysis was performed using non-parametric Mann-Whitney or Kruskal-Wallis test. Significance levels were set at $P < 0.05$.

Results

Scopolamine has been previously reported to produce a dose-dependent antidepressant-like effects in the forced-swim test (FST) in both rats and mice [7]. Here, we observed in CD-1 male mice such a dose-dependent efficiency in the TST (Kruskal-Wallis test, K=8.17, P=0.04; Figure 1A) with the only significant effect at a dose of 0.3 mg/kg (Mann-Whitney test, U=56.5, P=0.02). As observed for CD-1 mice, scopolamine significantly reduced in a dose-dependent manner the immobility of H/Rouen males (Kruskal-Wallis test, K=20.48, P=0.0001; Figure 1B) and females (Kruskal-Wallis test, K=18, P=0.0004; Figure 1C) in the TST. Interestingly, the effect was observed for both male and female mice at a dose as low as 0.03 mg/kg (Mann-Whitney test, $U_{males}=7$, $P_{males}=0.007$ and $U_{females}=0$, $P_{females}=0.002$), suggesting a higher sensitivity of H/Rouen mice than CD-1 mice to test antidepressant medications.

We then used NBQX to antagonize the effect of scopolamine in H/Rouen male mice. Again, scopolamine significantly decreased the immobility time in the TST at a dose of 0.1 mg/kg both in males (Mann-Whitney test, U=7; P<0.0001; Figure 2A) and females (Mann-Whitney test, U=3; P<0.0001; Figure 2B). Although a small effect still persisted when male and female mice first received an injection of NBQX before the injection of scopolamine (Mann-Whitney test, U_{males}=17; P_{males}=0.01; $U_{females}$=11; $P_{females}$=0.001), this effect was reduced for both sexes (Mann-Whitney test, U_{males}=19; P_{males}=0.007; $U_{females}$=26; $P_{females}$=0.04) when compared with treatment with scopolamine alone. As expected, no effect was observed after injection of NBQX alone for none of the genders (Mann-Whitney test, U_{males}=36; P_{males}=0.53; $U_{females}$=23; $P_{females}$=0.15; Figure 2).

Discussion

In this study, we examined the effect of scopolamine on mice with an extreme phenotype in the TST. We confirmed that scopolamine is effective in our mouse model of depression at very low doses. Scopolamine has previously been shown to increase the mobility time in rats and mice during FST [7, 10] and TST [7, 14]. Here, we observed, both in males and females, an effect on depressive-like phenotype at a dose ten folds lower than those previously published, suggesting that our H/Rouen model was a more sensitive model than commercial mice. In basal conditions, H/Rouen mice display an extremely long immobility duration in the TST with a low variability between individuals. In contrast, CD-1 mice are a heterogenous group of animals, including some having depressive-like behaviors whereas others have not, and for which scopolamine will have no antidepressant-like effect at low dose. The extreme homogeneous behaviors observed in our H/Rouen model is closer to a subgroup of

patients sharing similar phenotypes, as those observed in patients with major depressive disorders, and thus more appropriate to test antidepressant medications.

The antidepressant effect of scopolamine has been suggested to act through a rapid increase of the mammalian target of rapamycin (mTOR) complex 1 (mTORC1) signaling after a glutamate burst in the prefrontal cortex, as observed after the ketamine injection [10]. Consistent with previous reports [4, 10, 15], we observed that NBQX reduced the effect of scopolamine in resigned animals. This confirms that the antidepressant-like effects of scopolamine resulted from a glutamatergic burst [4]. However, this effect was not fully reversed by NBQX, in contrast to what has been previously reported in rats [10]. This suggests that in H/Rouen mice scopolamine might act through more than one mechanism. An additional action on noradrenergic transmission has been previously proposed [14] and may explain this partial effect. Indeed, a 50% reduction of noradrenaline in the prefrontal cortex, while not changing the mouse behavior in the TST, significantly reduce the higher mobility observed after scopolamine injection. It has also been proposed that dopamine D2/D3 receptors are involved in the rapid antidepressant-like effects of ketamine in the forced-swim test, since this effect was blocked by dopamine D2/D3 receptor antagonists [16]. Recently, Addy and colleagues showed that injection of scopolamine in the ventral tegmental area (VTA) decreased the immobility time in the FST [17], strongly supporting a role of VTA cholinergic activity in depressive-like behaviors. The blockade of the muscarinic acetylcholine receptors in the VTA by scopolamine may thus reduce the phasic dopamine release in the nucleus accumbens and modulate depressive-like behaviors through dopamine D2/D3 receptors. Scopolamine has also been shown to rescue depressive-like behaviors and increase the brain derived neurotrophic factor (BDNF), the serotonin transporter and the tryptophan hydroxylase 1 levels in reserpine-induced

mouse model of depression [18]. The BDNF increase after low dose of scopolamine injection is inhibited by L-Type Voltage-Dependent Calcium Channel antagonists and prevent the antidepressant-like effect of scopolamine in the FST [19]. These calcium channels have been repeatedly associated with mood disorders in human and are involved in the release of many neurotransmitters, as well as in the activity of midbrain dopaminergic neurons and adrenal chromaffin cells [20]. These mechanisms might thus explain the remaining antidepressant-like effect of scopolamine after NBQX injection observed in H/Rouen animals and need further experiments to decipher the molecular mechanisms involved in the antidepressant-like effect of scopolamine.

Conclusion

Overall, the present study shows evidence that the H/Rouen male and female mice are relevant models for preclinical studies to test new drugs alternative to standard antidepressant in treatment of major depressive disorder. In the future, such models should help in a better comprehension of the pathophysiology of depression.

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Conflict of interest

All other authors declare that they have no conflicts of interest.

References

[1] Slattery DA, Cryan JF. The ups and downs of modelling mood disorders in rodents. ILAR J. (2014) **55** 297-309.

[2] Malhi GS, Mann JJ. Depression. Lancet. (2018) **392** 2299-312.

[3] O'Leary OF, Dinan TG, Cryan JF. Faster, better, stronger: towards new antidepressant therapeutic strategies. Eur J Pharmacol. (2015) **753** 32-50.

[4] Duman RS. Ketamine and rapid-acting antidepressants: a new era in the battle against depression and suicide. F1000Res. (2018) **7**.

[5] Witkin JM, Martin AE, Golani LK, Xu NZ, Smith JL. Rapid-acting antidepressants. Adv Pharmacol. (2019) **86** 47-96.

[6] Podkowa K, Podkowa A, Salat K, Lenda T, Pilc A, Palucha-Poniewiera A. Antidepressant-like effects of scopolamine in mice are enhanced by the group II mGlu receptor antagonist LY341495. Neuropharmacology. (2016) **111** 169-79.

[7] Witkin JM, Overshiner C, Li X, Catlow JT, Wishart GN, Schober DA, et al. M1 and m2 muscarinic receptor subtypes regulate antidepressant-like effects of the rapidly acting antidepressant scopolamine. J Pharmacol Exp Ther. (2014) **351** 448-56.

[8] Cryan JF, Holmes A. The ascent of mouse: advances in modelling human depression and anxiety. Nat Rev Drug Discov. (2005) **4** 775-90.

[9] Cryan JF, Mombereau C, Vassout A. The tail suspension test as a model for assessing antidepressant activity: review of pharmacological and genetic studies in mice. Neurosci Biobehav Rev. (2005) **29** 571-625.

[10] Voleti B, Navarria A, Liu RJ, Banasr M, Li N, Terwilliger R, et al. Scopolamine rapidly increases mammalian target of rapamycin complex 1 signaling, synaptogenesis, and antidepressant behavioral responses. Biol Psychiatry. (2013) **74** 742-9.

[11] El Yacoubi M, Bouali S, Popa D, Naudon L, Leroux-Nicollet I, Hamon M, et al. Behavioral, neurochemical, and electrophysiological characterization of a genetic mouse model of depression. Proc Natl Acad Sci U S A. (2003) **100** 6227-32.

[12] El Yacoubi M, Vaugeois JM. Genetic rodent models of depression. Curr Opin Pharmacol. (2007) **7** 3-7.

[13] El Yacoubi M, Rappeneau V, Champion E, Malleret G, Vaugeois JM. The H/Rouen mouse model displays depression-like and anxiety-like behaviors. Behav Brain Res. (2013) **256** 43-50.

[14] Palucha-Poniewiera A, Podkowa K, Lenda T, Pilc A. The involvement of monoaminergic neurotransmission in the antidepressant-like action of scopolamine in the tail suspension test. Prog Neuropsychopharmacol Biol Psychiatry. (2017) **79** 155-61.

[15] Martin AE, Schober DA, Nikolayev A, Tolstikov VV, Anderson WH, Higgs RE, et al. Further Evaluation of Mechanisms Associated with the Antidepressantlike

Signature of Scopolamine in Mice. CNS Neurol Disord Drug Targets. (2017) **16** 492-500.

[16] Li Y, Zhu ZR, Ou BC, Wang YQ, Tan ZB, Deng CM, et al. Dopamine D2/D3 but not dopamine D1 receptors are involved in the rapid antidepressant-like effects of ketamine in the forced swim test. Behav Brain Res. (2015) **279** 100-5.

[17] Addy NA, Nunes EJ, Wickham RJ. Ventral tegmental area cholinergic mechanisms mediate behavioral responses in the forced swim test. Behav Brain Res. (2015) **288** 54-62.

[18] Yu H, Lv D, Shen M, Zhang Y, Zhou D, Chen Z, et al. BDNF mediates the protective effects of scopolamine in reserpine-induced depression-like behaviors via up-regulation of 5-HTT and TPH1. Psychiatry Res. (2019) **271** 328-34.

[19] Yu H, Li M, Shen X, Lv D, Sun X, Wang J, et al. The Requirement of L-Type Voltage-Dependent Calcium Channel (L-VDCC) in the Rapid-Acting Antidepressant-Like Effects of Scopolamine in Mice. Int J Neuropsychopharmacol. (2018) **21** 175-86.

[20] Andrade A, Brennecke A, Mallat S, Brown J, Gomez-Rivadeneira J, Czepiel N, et al. Genetic Associations between Voltage-Gated Calcium Channels and Psychiatric Disorders. Int J Mol Sci. (2019) **20**.

Figure legends

Figure 1. Dose-dependent effect of scopolamine. Increasing doses of scopolamine (0.03, 0.1 and 0.3 mg/kg) was injected intraperitoneally in 15 CD-1 males (A), as well as in 8 male (B) and 6 female (C) H/Rouen mice and compare to the vehicle injection. Immobility time during the tail suspension test was measure for 6 min, 30 min after injection. Individual measures are shown with black circles. Boxplots represent median ± interquartile range. **P<0.01, ***P<0.001.

Figure 2. Inhibition of the scopolamine antidepressant-like effect using the AMPA receptor antagonist NBQX. NBQX was injected or not in at least 7 male (A) and 8 female (B) H/Rouen mice at a dose of 10 mg/kg, 15 min before injection scopolamine (scop). Immobility times in a 6 min session of the tail suspension test was compared to vehicle injection (stars) or after injection of scopolamine (hashes). Individual measures are shown with black circles. Boxplots represent median ± interquartile range. *P<0.05, ***P<0.001, ##P<0.01.