

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

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

3

4 Running title: Scopolamine in H/Rouen mice.

5

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19

20 **Abstract**

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

34

35

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

38

39

40 **Introduction**

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

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

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

66

67 **Experimental procedures**

68 **Animals**

69 H/Rouen mice have been bred in the University Lyon 1 animal facility and selected for
70 their low mobility in the TST as previously described [11]. They were kept on a 7 a.m.-
71 7 p.m. light cycle with food and water ad libitum. Testing was performed between 9
72 a.m. and 5 p.m.. All experiments were conducted in accordance with the regulations
73 of the local ethical committee (Lyon 1 University CE2A- UCBL BH 2009-03) and the
74 European Community Council Directive of November the 24th, 1986 (86/609/EEC).

75

76 **Drugs**

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

80

81 **Tail Suspension Test**

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

86

87

88

89 **Drugs treatments**

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

95

96 **Statistical Analysis**

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

100

101 **Results**

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

113 We then used NBQX to antagonize the effect of scopolamine in H/Rouen male mice.
114 Again, scopolamine significantly decreased the immobility time in the TST at a dose of
115 0.1 mg/kg both in males (Mann-Whitney test, $U=7$; $P<0.0001$; Figure 2A) and females
116 (Mann-Whitney test, $U=3$; $P<0.0001$; Figure 2B). Although a small effect still persisted
117 when male and female mice first received an injection of NBQX before the injection of
118 scopolamine (Mann-Whitney test, $U_{\text{males}}=17$; $P_{\text{males}}=0.01$; $U_{\text{females}}=11$; $P_{\text{females}}=0.001$),
119 this effect was reduced for both sexes (Mann-Whitney test, $U_{\text{males}}=19$; $P_{\text{males}}=0.007$;
120 $U_{\text{females}}=26$; $P_{\text{females}}=0.04$) when compared with treatment with scopolamine alone. As
121 expected, no effect was observed after injection of NBQX alone for none of the genders
122 (Mann-Whitney test, $U_{\text{males}}=36$; $P_{\text{males}}=0.53$; $U_{\text{females}}=23$; $P_{\text{females}}=0.15$; Figure 2).

123

124 **Discussion**

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

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

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

171

172 **Conclusion**

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

177

178

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184 and the Bio-Psy Laboratory of Excellence.

185

186 **Conflict of interest**

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

188

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244 Disorders. *Int J Mol Sci*. (2019) **20**.

245

246

247 **Figure legends**

248 **Figure 1. Dose-dependent effect of scopolamine.** Increasing doses of scopolamine
249 (0.03, 0.1 and 0.3 mg/kg) was injected intraperitoneally in 15 CD-1 males (A), as well
250 as in 8 male (B) and 6 female (C) H/Rouen mice and compare to the vehicle injection.
251 Immobility time during the tail suspension test was measure for 6 min, 30 min after
252 injection. Individual measures are shown with black circles. Boxplots represent
253 median \pm interquartile range. **P<0.01, ***P<0.001.

254

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

262