Caffeine Mitigates the Locomotor Hyperactivity in Middle-aged Low-density Lipoprotein Receptor (LDLr)-Knockout Mice

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Received 29 January 2016; revision 22

February 2016; accepted 6 March 2016

doi: 10.1111/cns.12544

The low-density lipoprotein receptor (LDLr) plays a crucial role in the regulation of cholesterol levels in the blood by mediating the vascular uptake and intracellular degradation of low-density lipoprotein (LDL). Accordingly, the decrease in the activity of LDLr caused by genetic polymorphisms has been associated with an increased risk of myocardial infarction in humans [1]. Nevertheless, the hypercholesterolemia induced by LDLr deficiency (i.e., familial hypercholesterolemia) does not seem to affect only the peripheral system, given that recent clinical evidence has found an association between familial hypercholesterolemia and mild cognitive impairment [2]. In this regard, the use of LDLr-knockout mice (LDLr^{-/-}), an experimental model of familial hypercholesterolemia, has shed some light on the consequences of this condition in the central nervous system. Elder and colleagues demonstrated that genetic deletion of the LDLr in mice induces locomotor hyperactivity, suggesting that LDLr controls a general level of motor tone [3]. Moreover, spatial and working memory deficits in mice lacking the LDLr have been previously described in the literature [4].

The neuromodulator adenosine acts through four types of receptors, A₁, A_{2A}, A_{2B} and A₃ receptors. While the adenosine receptors are involved in a myriad of functions, the control of synaptic function, memory, exploratory behavior, and emotional function are highlighted [5]. Caffeine, the most widely used psychotropic drug in the world, acts mainly by the blockade of the A₁ and A_{2A} receptors with a discrete effect on the A_{2B} receptor [6]. Among the benefits of caffeine on brain function is the improvement of learning and memory processes and attenuation of age-related cognitive decline. Interestingly, some reports have identified that the effects of caffeine in rodents are optimal

depending mainly on the age [7]. Probably, this age-dependent effect induced by caffeine is related to the increase in the density of A_{2A} receptors during aging since the level of the other adenosine receptors remains unchanged [8].

Here, we hypothesize that caffeine counteracts the general locomotor hyperactivity exerted by LDLr deficiency in mice. To prove this idea, 3- and 8-month-old (m.o.) C57Bl/6 wild-type and LDLr^{-/-} female mice were submitted to a chronic (21 days) treatment via oral gavage with caffeine (10 mg/kg) and probe for spontaneous locomotor activity on the open-field arena on the next day. The daily administrations (caffeine or tap water) were performed at the beginning of the dark cycle to avoid the acute and withdrawal effects of caffeine on the behavioral analysis (performed during the light cycle, between 9:00 AM and 2:00 PM).

Reinforcing previous findings [3,4], we found increased locomotor activity in the LDLr^{-/-} mice. Specifically, LDLr^{-/-} mice at both ages showed increased total distance travelled in comparison with age-matched C57Bl/6 mice in the open-field test [genotype factor in 3 m.o.: $F_{(1, 38)} = 41.57$; $P < 0.05$; and 8 m.o.: $F_{(1, 47)} = 12.47$; $P < 0.05$] (Figures 1A and 2A). The hyperlocomotion was attenuated by the administration of caffeine selectively in 8 m.o. LDLr^{-/-} mice (Figure 2A) [interaction factor between genotype vs. treatment: $F_{(1, 47)} = 1.807$; $P < 0.05$]. The minute-by-minute analysis of the locomotor activity revealed a similar pattern of locomotion during the 5 min of the open-field test regardless of the genotype, treatment, or age (Figure 1B and 2B). In general, it can be observed that LDLr^{-/-} mice travelled higher distances than the respective wild-type mice during the entire period (5 min) of analysis. Interestingly, the treatment with caffeine induced a renormalization effect in the locomotor activity of

Figure 1 Genetic deletion of LDLr increases the locomotor activity in 3 m.o. mice as concluded from the total distance travelled (**A** and **B**), but does not interfere with the amount of time spent in the center of the open field (**C**). C57Bl/6 vehicle- (**D**) C57Bl/6 caffeine- (**E**), LDLr^{-/-} vehicle- (**F**) and LDLr^{-/-} caffeine- (**G**) treated mice display a similar thigmotaxic exploratory behavior. Data are expressed as mean $\pm$ SEM of 6–8 female mice per group. [#] $P < 0.05$ indicates a genotype effect (Newman-Keuls post hoc test).

Figure 2 Genetic deletion of LDLr increases the locomotor activity, which was attenuated by chronic treatment with caffeine (10 mg/kg, p.o.) in 8 m.o. mice. Increased total distance travelled by LDLr^{-/-} mice (**A** and **B**) but similar amount of time spent in the center (**C**). Similar thigmotaxic exploratory behavior between C57Bl/6 vehicle- (**D**) C57Bl/6 caffeine- (**E**), LDLr^{-/-} vehicle- (**F**) and LDLr^{-/-} caffeine- (**G**) treated mice. Data are expressed as mean $\pm$ SEM of 6–8 female mice per group. ^{*} $P < 0.05$ compared to wt/vehicle group; [#] $P < 0.05$ compared to LDLr^{-/-}/vehicle group (Newman-Keuls post hoc test).

8 m.o. mice. The analysis of the anxiety-like profile showed that all animal groups spent similar amount of time in the center of the open-field apparatus (Figures 1C and 2C). The visual inspection of the occupation plot suggests that the exploratory behavior is similar between groups respecting the age-dependent modifications in the exploratory behavior. Figure 1D–G revealed similar thigmotaxis and apparatus exploration pattern in 3 m.o. wild-type

and LDLr^{-/-} mice. Figure 2D–G also show equal exploratory behavior and thigmotaxis in 8 m.o. wild-type and LDLr^{-/-} mice.

Although the cholesterol levels are 3–4 times higher in LDLr^{-/-} mice, the effects mediated by caffeine are not directly related with the lipid profile since we did not observe differences in the plasma content of lipids in caffeine-treated C57Bl/6 and LDLr^{-/-} mice (data not shown). Caffeine as an antagonist of adenosine A₁R/A_{2A}

receptors has the ability to fine-tune the neural communication and to re-organize neural networks depending on the necessity of the system [9]. For this reason, it has been hypothesized that the more prominent effects of caffeine and adenosine receptors antagonists are related to disease conditions [9]. General hyperactivity is a recognized phenotype following LDLr deficiency in mice [3]. Our results indicate that caffeine is not able to modify the hyperlocomotion observed in 3 m.o. LDLr^{-/-} mice. However, caffeine attenuated the increased locomotor activity observed in 8 m.o. LDLr^{-/-} mice. Interestingly, preclinical studies have shown that caffeine effects are optimal depending on the age of the animals and the parameter analyzed [7]. The age-dependent effect induced by caffeine is in line with the idea that the blockade of adenosine A₁/A_{2A} receptors attempts to renormalize a potentially maladapted system [6], considering age as an important escalating factor in LDLr^{-/-} mice. In agreement, evidence from our research group further suggests that genetic deletion of LDLr in mice accelerates some age-related features in the brain, such as oxidative damage and alterations of cholinergic signaling [4].

In conclusion, this is the first report demonstrating that young (3 m.o.) and middle-aged (8 m.o.) LDLr^{-/-} mice display differ-

ent responses to chronic caffeine treatment, at least in terms of motor activity. As caffeine is widely consumed, the individual variability and selective outcomes from its consumption are certainly of interest. However, further studies are necessary to better characterize which synaptic alterations are responsible for this modification in the pattern of caffeine effects. Finally, microglia and astrocytes release important factors for neuronal survival and growth after stimulation via adenosine receptors [10]; thus, the investigation of the role these mechanisms in age-dependent caffeine effects represents a very exciting new field of research.

Acknowledgments

The authors are thankful to Dr. Rodrigo Medeiros for revision of this manuscript and the Brazilian agencies CNPq, CAPES and FAPESC for the financial support.

Conflict of Interest

The authors declare no conflict of interest.

References

- Thormahlen AS, Schuberth C, Won HH, et al. Systematic cell-based phenotyping of missense alleles empowers rare variant association studies: A case for LDLR and myocardial infarction. *PLoS Genet* 2015;**11**: e1004855.
- Zambon D, Quintana M, Mata P, et al. Higher incidence of mild cognitive impairment in familial hypercholesterolemia. *Am J Med* 2010;**123**:267–274.
- Elder GA, Ragnauth A, Dorr N, et al. Increased locomotor activity in mice lacking the low-density lipoprotein receptor. *Behav Brain Res* 2008;**191**: 256–265.
- Moreira EL, de Oliveira J, Nunes JC, et al. Age-related cognitive decline in hypercholesterolemic LDL receptor knockout mice (LDLr^{-/-}): Evidence of antioxidant imbalance and increased acetylcholinesterase activity in the prefrontal cortex. *J Alzheimers Dis* 2012;**32**: 495–511.
- Cunha RA. Adenosine as a neuromodulator and as a homeostatic regulator in the nervous system: Different roles, different sources and different receptors. *Neurochem Int* 2001;**38**:107–125.
- Fredholm BB. Adenosine and neuroprotection. *Int Rev Neurobiol* 1997;**40**:259–280.
- Poole RL, Braak D, Gould TJ. Concentration- and age-dependent effects of chronic caffeine on contextual fear conditioning in C57BL/6J mice. *Behav Brain Res* 2016;**298**:69–77.
- Canas PM, Duarte JM, Rodrigues RJ, et al. Modification upon aging of the density of presynaptic modulation systems in the hippocampus. *Neurobiol Aging* 2009;**30**:1877–1884.
- Sebastiao AM, Ribeiro JA. Adenosine receptors and the central nervous system. *Handb Exp Pharmacol* 2009;**193**:471–534.
- Matos M, Shen HY, Augusto E, et al. Deletion of adenosine A2A receptors from astrocytes disrupts glutamate homeostasis leading to psychomotor and cognitive impairment: relevance to schizophrenia. *Biol Psychiatry* 2015;**78**:763–774.