Reviewer's report

Title: Inhibition of adenosine A1 receptors abolished the nutritional ketosis-evoked delay in the onset of isoflurane-induced anesthesia in Wistar Albino Glaxo Rijswijk rats

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Reviewer: Li Ma

Reviewer's report:

The authors' previous study has shown that supplementation of nutritional ketones delays the onset of isoflurane-induced anesthesia in rats. In the current study, the authors expanded their study and demonstrated that the delaying effect of ketosis was abolished by blocking of adenosine A1 but not A2A receptors. The study is straightforward and very interesting. However, there are some concerns regarding the experimental design and manuscript discussion.

1. According to the manuscript, ketosis increases adenosine level [14] in the brain tissues and adenosine is closely associated with sleep and sleep-like state, including anesthesia. It is plausible that the authors investigate whether adenosine is involved in ketone-evoked delay of isoflurane-mediated anesthesia. Adenosine A1Rs are inhibitory and A2ARs are excitatory in the ventrolateral/lateral preoptic and basal forebrain, which areas are implicated in the generation of sleep and sleep-like effects [28]. It is easy to understand that stimulation of A1R (inhibitory) is one of the mechanisms by which isoflurane produces anesthesia [49] and A1R specific agonist causes an increase in recovering time from anesthesia [48]. However, it is difficult to comprehend that inhibition of A1Rs (inhibition of inhibitory signaling which is supposed to help to stay awake) could abolish the delay of isoflurane-mediated anesthesia by ketosis. Before moving any further, I think the authors should include an experiment using animals that are not fed with ketones to see whether these agents themselves have any effects on the induction of isoflurane-induced anesthesia. [E.g, three groups: 1. standard diet (SD) alone; 2. SD + A1R antagonist; 3. SD + A2AR antagonist].

2. If these agents themselves do not have any effects on isoflurane-induced anesthesia but only abolish the ketosis-mediated delay of induction of anesthesia, more discussions on the interrelationships among ketones, adenosine A1Rs /A2ARs and anesthesia, as well as interpretations of the discrepancy and possible mechanisms should be included in the manuscript.

Are the methods appropriate and well described?
If not, please specify what is required in your comments to the authors.

Yes

Does the work include the necessary controls?
If not, please specify which controls are required in your comments to the authors.
No

**Are the conclusions drawn adequately supported by the data shown?**
If not, please explain in your comments to the authors.

Yes

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I am able to assess the statistics

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