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: Zheng Xie

Reviewer's report:

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Kovacs Z et al. reported that inhibition of adenosine A1 receptors abolished the nutritional ketosis-evoked delay in the onset of isoflurane-induced anesthesia in Wistar Albino Glaxo Rijswijk rats.

This group published the initial report on this topic in BMC anesthesiology in 2018, suggesting nutritional ketosis delays the onset of isoflurane induced anesthesia. In this article, the authors looked into the possible role of adenosine receptors in ketogenic diet induced delay onset of isoflurane induction in Wistar Albino Glaxo Rijswijk (WAG/Rij) rats, a model strain of human absence epilepsy.

The authors concluded that adenosine A1 receptors may be responsible for the ketosis-induced delay in the onset of isoflurane-induced anesthesia in this strain of rats. They also suggested that the delay effect correlated with blood β-hydroxybutyrate (βHB levels).

The finding of this study is a step forward from their previous publication with one of the possible mechanisms underlying the ketogenic diet induced delay of the induction of isoflurane anesthesia. The adenosine A1 receptor may play a significant role in this effect of ketogenic diets. The hypothesis and methods are reasonable.

There are a few main points which I would like the authors to address.

1. The authors used the term "immobility" to define the induction of anesthesia. If I understand it correctly, the authors defined the induction time as the start of 3% isoflurane to the animals stopped moving in the anesthesia chamber. In clinical anesthesia, immobility generally is referred as patients do not respond to noxious stimulation. Induction of anesthesia is referred as the induction of unconsciousness. In rodents, the induction of anesthesia is generally measured by the loss of righting reflex. Sometimes, the animals stop moving but still have righting reflex initially. The immobility is generally tested by tail clamping technique in rodents. The anesthesia level is in a deeper stage for immobility in both humans and rodents than the stage for the induction of unconsciousness. The sites (spinal cord vs brain) of actions and the
molecular mechanisms for the induction of unconsciousness and immobility may be different. It is likely the "immobility" measured in the article is under the lighter phase of anesthesia than the phase of "loss of righting reflex". The authors should provide the explanation of their definition "immobility" is different from the one used in the field of anesthesia. This is particularly important when they discuss the mechanisms underlying the induction of anesthesia. The authors should be cautious to refer this finding is relevant to the surgical phase of anesthesia in their abstract.

2. Like humans, each individual rat in the same group may respond to the same concentration of isoflurane differently. Crossover design cannot be done in this study. Showing the means of different groups did not show the variability within the group. The author should show the individual data of each rats in the histograms (Fig 1). Using SD, not SE, will also be helpful for readers to see the dispersion.

3. In table 3, the largest difference (44.25 sec) in mean between groups is group 8 and group 1. It is about 31% delay in the induction of "immobility". The results are statistically different. But it is relatively insignificant clinically. The study would be better if it includes the effects on the righting reflex and the emergence from anesthesia. In addition, the authors used in Wistar Albino Glaxo Rijswijk (WAG/Rij) rats in this study. The data would be stronger if similar experiments were also done in a normal strain, Sprague-Dawley rats which were used in their initial study. I understand the authors tried to explain the reason to use WAG/Rij in this study in the introduction. I do not mean to ask the authors to perform all these studies for this publication. However, it is important to discuss the limitations and future directions.

4. In table 1, the levels of blood ßHB were higher with the ketone supplement groups. The data suggested some positive correlation. However, the R value is relatively weak. It is correct for the author to claim some correlation, but not causation in their discussion.

5. The main finding of this article is the involvement of adenosinergic system in the induction of anesthesia. The authors suggested that exogenous ketone diets may affect adenosinergic system, which is subsequently responsible for the delay of in the onset of isoflurane-induced anesthesia in WAG/Rij rats. The authors tested A1 receptors and A2A receptors in their experiments. They showed that the effect of ketosis diets on the induction of anesthesia was completely abolished by DPCPX, an A1 receptor antagonist, but was affected by SCH 58261, an A2A receptor antagonist. While these antagonists were believed as selective antagonists, the concentrations used in this study were high and might not be selective. Was the same amount of DMSO given to both groups? In addition, only one concentration of each antagonist was administrated by IP. IP was not the best way to deliver the drugs accurately to determine the selectivity. With intravenous injection, the drugs may be delivered more efficiently and reliably with lower concentrations. In the literatures, both A1 and A2A receptors have been reported to be targets of anesthetics. The authors should explain about discrepancies and their conclusion in the discussion. Again, I would like to ask the authors should show the individual data in their histograms to show the variability within the group.
Can the authors measure adenosine levels in the brains of these rats after their behavioral study? If the ketogenic diets affect the adenosinergic system, do the authors have any plan to test other neurotransmitter systems which may play roles in the induction of anesthesia in animals fed with ketogenic diets? The recent review article by Kelz, M at al. in Anesthesia and Analgesia (128: 726-36, April, 2019) summarized the current findings in adenosinergic and other systems which may play important roles in the anesthetic-induced unconsciousness (probably both in the induction unconsciousness and the emergence from unconsciousness).

Minor points:

1. Line 158 on page 7, should the unpublished and preliminary results be disclosed? The full name of SWD should be written.

2. Please explain why gavage for 7 days was chosen in this study and the previous paper. Any evidence to suggest adenosine levels will go up after gavage for 7 days?

3. Can the authors explain why water gavage for 5 days (adaptation period) was used? It is good to have an adaptation period. Gavage alone is a stressful stimulant to animals. Stressful stimulation alone may change the sensitivity of these animals to anesthetics (Wang, L et al March 2019, PLOS one, https://doi.org/10.1371/journal.pone.0214093 )

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.

Yes

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

Yes

Are you able to assess any statistics in the manuscript or would you recommend an additional statistical review?
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