

The authors of the manuscript "Hyperglycemia Stimulates the Irreversible Catabolism of 2 Branched-Chain Amino Acids and Generation of Ketone Bodies by Cultured Human Astrocytes" have researched the features of the influence of hyperglycemia on the astrocyte release/uptake of compounds and metabolites from the culture medium. Since the number of patients with diabetes mellitus is currently steadily increasing, it is of undeniable interest to study the effect of hyperglycemia in uncompensated diabetes on the function of astrocytes. In this view, the presented manuscript has certain scientific interest.

Comments.

1. In the chapter "2. Materials and Methods" in 2.2. It is necessary to indicate the glucose concentration in the medium in which the cells are cultured.
2. It is not clear at what passage the cells were taken into the experiment, how many days were they cultured on the plate before the start of the experiment? With what concentration of cells and in what volume were they placed per well of a 6-well plate?
3. In "2.4. Protein Estimation" needs to clarify: What was the lysis buffer was used to receive cell lysate?
4. In "2.7. Statistical Analysis" the authors have written "...of six independent experiments..", It is necessary to clarify whether these were separate passages or plates, if passages, then which ones? The range or number of passages used in the study must be specified.
5. Lines 159-161: The authors have provided descriptions of the calculation of cell death using the LDH test. This information needs to be moved from chapter "3. Results" to the appropriate methods section.
6. In Table 1 on the page 5, the authors have written "Protein content (mg)." It is needed to clarify the value of measuring - mg per ml or per well?
7. Lines 34, 198: The authors have used the word "euglycemia", How is it different from "normoglycemia" (Lines 193, 213) It is necessary to use uniform terms and designations.
8. Lines 240-245 276, 286, 288: The authors have used the term "euglycemic", Please see remarks up (7.).
9. In Fig. 1 authors must correctly name the components of the legend. All three groups are culture medium, not 5mM/25mM glucose, as indicated in the figure legend.
11. It is necessary to make the changes in Fig. 2 to correctly reflect the data. In the caption to the figure and in the legend, it is necessary to clarify what 5 mM and 25 mM are - specifying that this is the glucose content in the culture medium or normo- or hyperglycemia.
12. The medium used in the work for culturing astrocytes contains both a specific amino acid composition and 10% serum, which has a complex composition. In this regard, have the authors analysed what happens to the composition of this medium when it is kept in an incubator for 24 hours in the absence of cells? Accordingly, as a control, it would be more correct to use a medium with a corresponding glucose concentration of 5 or 25 mM, which was kept in an incubator without cells for 24 hours. Since it is not clear how stable the composition of the medium is when kept for a long time at 37 degrees and 5% CO₂.

13. In the abstract, the authors have written: "The observed metabolic activities of astrocytes provide valuable insights into their roles in supporting neuronal function, brain metabolism, and intercellular metabolic interactions within the brain." However, the work did not study two-dimensional cultures, including both astrocytes and neurons, so it is not correct to talk about the state of neurons and intercellular interaction.