Role of the autonomous nervous system in the cerebral response to a low concentration of intragastric glucose perfusion in mice

Claude Knauf, Elias Mokhliss, Patrice D. Cani, Estelle Martinez, Xavier Fioramonti, Christophe Perrin, Miguel A. Iglesias, Rémy Burcelin

Introduction

During the post-prandial period, glucose absorbed in the duodenum is conducted to the liver via the hepatoportal vein. Glucose then enters the systemic circulation to reach the heart, from where it is distributed to other organs. Whole body glucose fluxes are controlled by numerous neuronal, hormonal and metabolic factors in order to maintain a normal glycaemia. Increases of glucose concentration are detected by specialized structures called glucose sensors. These structures are situated in different body locations, such as in the pancreas (ß-cells), in the hepatoportal vein and in the central nervous system. Sensor activation generates a peripheral physiological response, inducing glucagon secretion, alteration of food intake and tissue glucose utilisation, among others. The physiological response is produced by neuronal and hormonal signals, which inform organs implicated in the utilisation and/or production of glucose of the increased glycaemia. An example of these signals is the insulin released in response to an absorptive hyperglycemia.

In the present study we hypothesised that the gastro-intestinal tract may inform the brain of the presence of glucose in the gut, revealing a new glucose sensing mechanism that acts independently to the known glucose sensors in other parts of the organism.

Specific aims

The first aim of this work was to localize the cerebral regions activated in response to an intragastric perfusion of glucose. We speculated that a nervous signal may serve as relay between the gastro-intestinal glucose detection and the central nervous system. For this reason, we studied the expression of c-Fos, a marker of neuronal activity, in specific brain regions implicated in the glucose homeostasis, during an intragastric perfusion of glucose. The dose of glucose infused was too small to increase peripheral glycemia, avoiding the activation of systemic glucose. To confirm the importance of the autonomous nervous system in the gastro-cerebral axis, similar experiments were performed on mice treated by intragastric perfusion of capsaicin, compound that destroys the afferent nervous signal.

The last aim of this work was to measure the physiological responses to the gastro-intestinal glucose detection. We determined the glycaemia and insulinaemia during the experiments, as well as glucose fluxes in the whole body of mice using the isotopic dilution method.

Materials and methods

Surgery

See experimental protocol on figure 1.
Intragastric catheters were placed on the proximal stomach of 12 weeks-old c57bl6 mice (day 1).

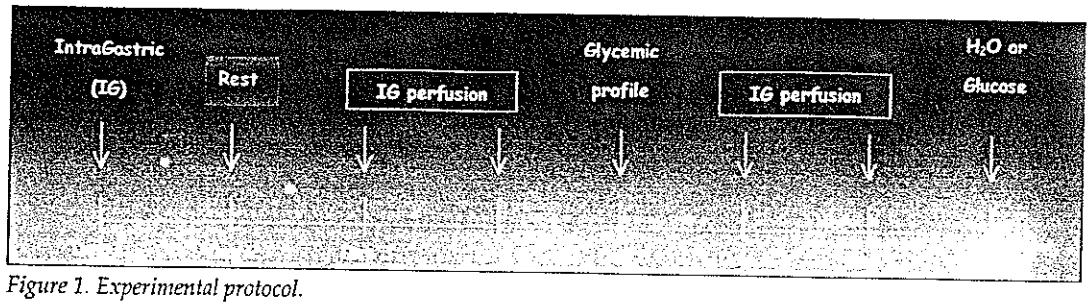

Figure 1. Experimental protocol.

Perfusion

After one day of rest (day 2), mice were perfused (100µl, 10 min/day, 2 consecutive days) with sterile water, to accustom them to the perfusion (days 3 and 4).

Glycaemic profile

On day 5 we studied the glycaemic profile in response to the glucose infusion. After a 6-hour fast, a small dose of glucose (100 mg/kg dissolved in 100 µl d'H_2O, infused in 10 min) was perfused into the stomach, and glycaemia was measured every 20 minutes for 2 hours.

Perfusion

Days 6 and 7 correspond to a new phase of accommodation of mice to be perfused (100 µl of sterile water, 10 minutes).

Physiological study (experiment day)

On day 8, 6-hour fasted mice were perfused with water or glucose (100mg/kg, 100 µl, in 10 minutes). A blood sample was taken two hours after the beginning of the infusion to measure glycaemia and insulinemia. Mice were then fixed with a mixture of paraformaldehyde and picric acid, and the brain was extracted to study c-Fos expression by immunohistochemistry.

Immunohistochemistry

Brains were post-fixed during one day, placed in a solution of 20 % of sucrose during 2 days and then cut in 35 µm-thick slices. Variations in c-Fos expression was determined in four specific brain regions: Three hypothalamic regions (arcuate nucleus, dorsomedian hypothalamus and ventromedian hypothalamus) and one region of the brain stem, the nucleus of the tractus solitarius, a region known as an intermediate structure between the periphery and the brain. c-Fos positives cells were counted in every other slice for each whole region.

Capsaicin treatment

Mice were treated by capsaicin perfusion through the stomach for 3 days (days 5, 6 and 7 of protocol) before the experiment day (day 8) at a dose of 10 mg/kg. Variations of c-Fos expression in the brain were then studied by immunohistochemistry as described above.

Glycaemia and insulinemia measurement

Plasma glucose was determined at the end of the infusion procedures using a glucose meter, GlucoTrend2 (Roche Diagnostic, Rotkreux, Switzerland, CH). Plasma insulin was determined by ELISA.

Glucose flux measurement

Whole body glucose flux was measured by the isotopic dilution method described previously (Perrin et al., Endocrinology, 2004). Calculations for the determination of glucose utilization were made from parameters obtained during the last 60 minutes of the infusions in steady state conditions. Briefly, the D-(3H)3-glucose specific activity was calculated by dividing the D-(3H)3-glucose enrichment by the plasma glucose concentration. The whole body glucose turnover rate, corresponding to the glucose utilization, was calculated by dividing the rate of D-(3H)3-glucose by the D-(3H)3-glucose plasma specific acti-

vity. The whole body glycolytic flux was calculated from the $3H_2O$ accumulated in the plasma during the last hour of the infusions. The whole body glycogen synthesis rate was calculated by subtracting the glycolytic flux from the glucose turnover rate. For each mouse the mean values have been calculated and averaged with values from mice of the same group. Mice showing variations of the steady state D-(3H)3-glucose specific activity larger than 15% during this time period were excluded from the study.

Results

Glycemic profile (day 4)

The intragastric glucose perfusion (100mg/kg during 10 minutes, 100µl) did not increase the peripheral glycaemia during the 2 hours preceding the glucose injection. Therefore, this dose of glucose is unlikely to activate the known glucose sensors cited previously.

Glycaemia and insulinaemia (day 8)

After 2 hours of experiment, no significant variations of glycaemia and insulinaemia were observed in mice treated with glucose or sterile water.

Expression of c-Fos in the hypothalamus and in the brain stem

The intragastric perfusion of glucose caused a significant decrease in the number of c-Fos positive cells in the arcuate nucleus (figure 2), in the dorsomedian hypothalamus and in the ventromedian hypothalamus. An inverse mode of regulation is observed in the nucleus of the tractus solitarius where the number of c-Fos positives cells increase significantly in response to the intragastric glucose treatment. These effects are a specific response to glucose, since different c-Fos expression profile was observed in the brain of mice infused with mannitol.

Expression of c-Fos in the hypothalamus and in the brain stem of mice treated with capsaicin

The presence of capsaicin in the gastro-intestinal tract totally abolished the glucose effect in the brain (figure 2), demonstrating the importance of the autonomous nervous system in the relay of the gastro-intestinal signal to the brain.

Glucose flux measurement

These results demonstrate that intragastric glucose perfusion increased significantly the glucose utilization in the whole body of mice. This increase in glucose utilization is due to an increase in glycolysis and glycogen synthesis (figure 3).

Conclusion

Our results show that small dose of glucose in the gut may be detected by the gastro-intestinal tract, which sends a message to the brain via the autonomous nervous system, to signal the presence of glucose. As a result, neurons in the hypothalamus decrease their activity.

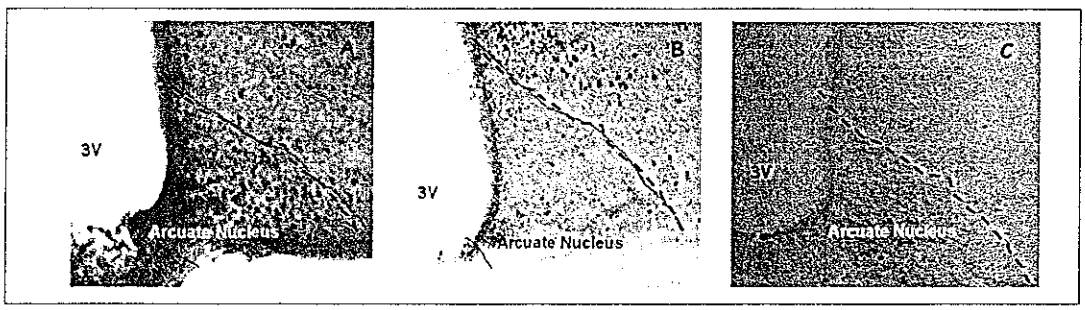

Figure 2. c-Fos expression in the arcuate nucleus of mice treated with sterile water (A), glucose (B) or glucose with capsaicin (C).

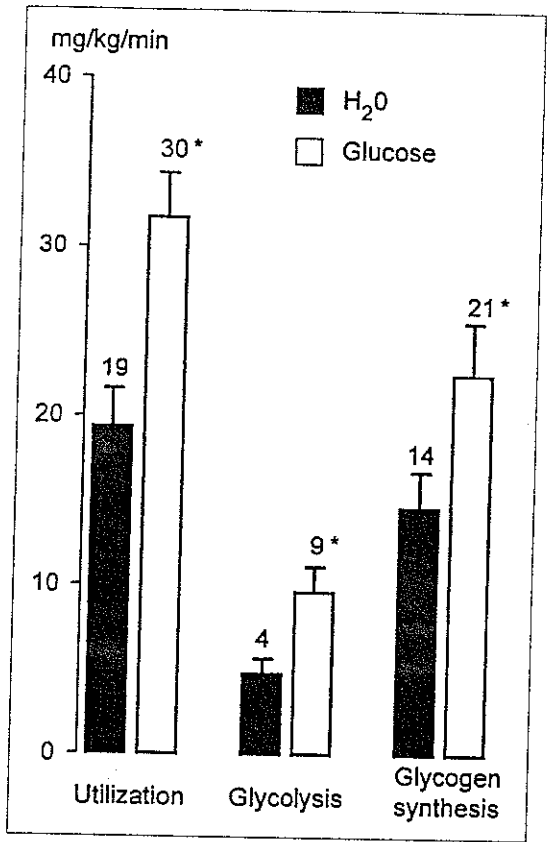

Figure 3. Whole body glucose utilization of mice treated with sterile water of glucose.

The consequence of modifications of neuronal activity in the brain stem and in the hypothalamus is an increase in glucose flux in the whole body. The future aim of our work is to identify the neuronal population implicated in the signal pathway.

References

- Perrin C., Knauf C., Burcelin R. *Intracerebroventricular infusion of glucose, insulin, and the adenosine monophosphate-activated kinase activator, 5-aminoimidazole-4-carboxamide-1-beta-D-ribofuranoside, controls muscle glycogen synthesis.* Endocrinology, 145 (9), 4025-4033, 2004.

The author

Claude Knauf, PhD
CHU Rangueil - Bâtiment L1
CNRS UMR 5018
TSA 50032
31059 Toulouse Cedex 9 - France
e-mail : claude.knauf@toulouse.inserm.fr