Acta Physiol Scand 2001, **173**, 223–230

Exercise-induced changes in brain glucose and serotonin revealed by microdialysis in rat hippocampus: effect of glucose supplementation

F. BÉQUET, D. GOMEZ-MERINO, M. BERTHELOT and C. Y. GUEZENNEC

Department of Physiology, IMASSA, Brétigny-sur-Orge Cedex, France

ABSTRACT

The aim of this study was to assess extracellular glucose changes in hippocampus in response to physical exercise and to determine the influence of glucose supplementation. In the same time, we have observed the changes in serotonin, in order to study the relationship between glucose and serotonin during exercise. Both glucose and serotonin were assessed using microdialysis. Exercise induced an increase in extracellular glucose levels over baseline during exercise to 121.1 ± 3.0% ($P < 0.001$), then a decrease to baseline during recovery. The serotonin followed glucose changes during the first 90 min of exercise, but followed a different pattern during recovery, increasing to a maximum of 129.9 ± 7.0% after 30 min of recovery ($P < 0.001$). When a 15% glucose solution was infused (10 μL min^{-1}) during exercise and recovery, blood glucose concentration was increased, but extracellular brain glucose decreased to reach a minimum of 73.3 ± 4.6% after 90 min of recovery ($P < 0.001$). Serotonin was always the mirror-reflect of cerebral glucose, with a maximum increase of 142.0 ± 6.9% after 90 min of recovery ($P < 0.001$). These results show that exercise induces changes in brain glucose and 5-hydroxytryptamine (5-HT) levels, which were dramatically modified by glucose infusion. Taking into account the implication of brain 5-HT in central fatigue, they suggest that if glucose supplementation, before and during exercise, undoubtedly increase performance because of its peripheral positive action, it would have a negative impact on the quality of recovery after the end of the exercise.

Keywords exercise, glucose, hippocampus, microdialysis, serotonin.

Received 9 October 2000, accepted 26 March 2001

Factors of peripheral fatigue, such as blood glucose and muscle glycogen lowered levels, have been well documented (Gibson & Edwards 1985). Early research by Levine in the 1920s established the link between hypoglycaemia and extreme fatigue (Levine *et al.* 1924). Later, works performed in Scandinavia (Bergström & Hultman 1967, Saltin & Karlsson 1971) revealed the existence of a link between fatigue and peripheral glycogen utilization. However, all fatigue-related phenomena cannot be explained by this sole observation. Since then, it has also been shown that fatigue can occur from changes in the central nervous system (CNS) (Bigland-Ritchie *et al.* 1986). Chaouloff *et al.* (1985) have shown that prolonged exercise could induce alterations in neurotransmitters functions, via the brain serotonergic system and Newsholme *et al.* (1987) were the first to relate the hypothesis of a 'central fatigue' to the activity of the serotonin (5-HT: 5-hydroxytryptamine). Its role has been evidenced by Bailey *et al.* (1993a) in animal studies, showing that performance during a prolonged exercise was enhanced by the administration of a 5-HT antagonist. In the same way, they have observed an increase in the concentration of 5-HT in various regions of the brain after a prolonged exercise of 1 h and after an exercise to exhaustion (Bailey *et al.* 1993b). Recently, a study performed at the laboratory, has shown that an acute intensive exercise induced a significant increase of 5-HT levels in cortex and hippocampus (Gomez-Merino *et al.* 2001).

Some studies on muscular fatigue have shown that glucose supplementation can delay fatigue induced by physical exercise or pointed out that supplementing carbohydrates during exercise significantly lengthened its duration (Coyle *et al.* 1986, Neufer *et al.* 1987). Experiments carried out by Koslowski *et al.* (1981) on dogs, where glucose was directly infused in the carotid artery, showed that supplying glucose to the brain could delay fatigue, therefore hinting towards an

Correspondence: F. Béquet, Department of Physiology, IMASSA – B.P. 73, 91223 Brétigny-sur-Orge Cedex, France.

action on fatigue of peripheral glucose in the brain. More recently, we have shown that an exercise to exhaustion increased extracellular brain glucose metabolism in spite of hypoglycaemia, suggesting a specific regulation of cerebral glucose after exercise (Béquet *et al.* 2000). These data suggest that glucose could play a specific role in the brain during physical exercise, in addition to its well known peripheral action. It also has been shown that the brain glucose availability could influence 5-HT and amino acids such as GABA (γ-aminobutyric acid) or glutamate metabolisms (Wong & Tyce 1983, Vahabzadeh *et al.* 1995). Fernstrom & Wurtman (1971) have observed an increase in brain serotonin synthesis following a high carbohydrate diet and Vahabzadeh has observed a mirror effect between extracellular hippocampal glucose and 5-HT variations under the influence of blood glucose variations: an insulin injection induced a decrease of brain glucose and an increase of 5-HT, then a subsequent glucose infusion induced an increase of brain glucose and a decrease of 5-HT. More recently, Maekawa *et al.* have observed the presence of GK-like enzyme in serotonergic neurones, which could be a glucose-sensing unit, confirming a probable influence of brain glucose on the serotonergic system. In parallel, a study performed by Pennington & Pentreath (1987) showed a regulation of glycogen metabolism in brain by some neurotransmitters (5-HT, GABA, dopamine, noradrenaline), the 5-HT activating glycogen catabolism. This observation was confirmed by Poblete & Azmitia (1995), who showed an activation of GPase by 5-HT. All these data show a complex interaction system between brain glucose and 5-HT. However, it seems that exercise could modify brain glucose metabolism and, as a consequence, influence the serotonergic system, underlying a possible relation between peripheral glucose availability, cerebral glucose metabolism and this neurotransmitter.

In this study, we aimed first at determining if brain glucose metabolism was influenced by physical exercise and if these changes could be related to 5-HT response to exercise. To this end, we focused on the relationship between extracellular hippocampal glucose and 5-HT during an acute intensive exercise and compared these data with glycaemic changes. Then, we have monitored the effect of a glucose infusion during exercise on both metabolites in order to determine if changes in glycaemia could influence the brain glucose and 5-HT responses to exercise. We used the *in vivo* microdialysis technique, applied to the exercise conditions, which permitted to observe simultaneously 5-HT and glucose changes during all the time of the experiment. This technique was combined with a chronic venous catheterization in order to infuse glucose in the second protocol.

MATERIALS AND METHODS

Animals

Male Wistar rats (225–249 g) from Janvier (Le Genest-Saint Isle, France) were kept on a 12 : 12 h light–dark cycle, and were fed with a standard diet, *ad libitum*. On the day of experiment the rats weighed approximately 310 g. The protocols were approved by the veterinary surgeons of the IMASSA.

Surgery for simultaneous brain microdialysis and venous catheterization

Rats were anaesthetized with pentobarbital sodium (60 mg kg^{-1}), placed in a stereotaxic frame and microdialysis probe guide was implanted in the ventral hippocampus (5.2 mm posterior to bregma, 5.0 mm lateral to medline, and 3.6 mm ventral to dura) according to the atlas of Paxinos & Watson (1982).

To minimize stress in this *in vivo* study, a jugulo-cardiac venous catheterization was used for glucose supplementation in a second group. The indwelling venous catheter was implanted before probe guide, according to a previously described technique (Nicolaidis *et al.* 1974). Briefly, a silicone rubber catheter, filled with viscous polyvinylpyrrolidone solution (40% w/v), was inserted in the right auricular cavity via the right external jugular vein approximately 5 mm before it dives under the clavicle. The vein was tied around the catheter and held in place by further sutures. The other end of the catheter was pulled subcutaneously through a slit in the skin at the top of the skull.

The guide, the venous catheter and three screws were fixed to the skull with dental cement. An animal antibiotherapy was administered postoperatively, and we have waited 5 days for postoperative recovery.

Protocol training

After the recovery from surgery the rats were placed on a rodent treadmill five to six times to be gradually accustomed to run at 25 m min^{-1}. They exercised each time for 30 min. The day before microdialysis experimentation, the rats were able to run 30 min at 25 m min^{-1}. Animals reluctant to run, during this period of adaptation, were excluded.

Experimental procedures

Two groups of seven fed rats capable to run were randomized with or without glucose supplementation. Sampling of the dialysates were collected every 30 min.

After collecting at least four baseline dialysis samples, we began progressively the running. The

running speed of 25 m min^{-1} being reached, the dialysates samples were collected during exercise (120 min/four samples) and recovery (150 min/five samples). During recovery, the rats stayed on the treadmill with water availability.

In the second protocol, a 15% glucose (in NaCl 0.9%) solution infusion was performed during exercise and recovery at a rate of 10 μL min^{-1}. Pre-tests laboratory have shown that such an administration was sufficient to induce a significant increase in plasma glucose levels and to prevent exercise-induced hypoglycaemia, which was the aim of this glucose supplementation.

Microdialysis procedure

The day of the experimental situation, the microdialysis probe with a membrane length of 3 mm (CMA/12, Carnegie Medicin, Stockholm, Sweden) was inserted and the rats were placed on the treadmill. The probes were connected to a microinfusion pump CMA 102 (Carnegie Medicin) and infused with a modified Ringer's solution (119.5 mM NaCl, 4.75 mM KCl, 1.27 mM $CaCl_2$, 1.19 mM KH_2PO_4, 1.19 mM $MgSO_4$ and 1.6 mM Na_2HPO_4) at a constant flow rate of 2 μL min^{-1}. To minimize stress effect and to allow the establishment of stable levels of monoamine and glucose exchanges between the brain tissue and the dialysis medium, the rats stayed unexercised on the treadmill during at least 2 h. An adjustment was made to the treadmill in order to attach the counter-balance arm of the microdialysis system.

In the second protocol, the venous catheter was prolonged and fixed to the counter-balance arm.

Analysis of dialysate samples

Reverse-phase high-performance liquid chromatography (HPLC) with fluorometric detection was used for determination of 5-HT in dialysates. The system consisted of a 10-μL sample loop leading to an Eco-Cart Lichrospher RP-18 (5 μm; 75 × 4.0 mm) column (Merck, Eurolab Fontenay-Sous-Bois, France). The mobile phase consisted of a phosphate buffer 0.03 M (pH = 3) with 30% of MeOH. The detection was set at a 283-nm excitation wavelength and a 345-nm emission wavelength, with a Jasco FP-920 fluorometric detector. The 5-HT from chromatograms of dialysate samples was identified by comparing its elution time with those of reference standards. The limit of detection for 5-HT was 2.5 fmol (10 μL)$^{-1}$, sufficient to measure basal levels of 5-HT without the use of a re-uptake inhibitor added to the perfusion fluid.

Extracellular brain glucose was measured by means of a method coupling glucose oxydase and peroxydase (Glucose Enzymatique Color kit, Biotrol Diagnostic, Merck) with a spectrophotometric detection at 500 nm.

Histological analysis

At the end of each experiment, animals were killed and their brains removed. Brains were sectioned with a refrigerated cryostat, stained with Cresyl violet and inspected to determine the localization of the probe.

Glycaemia

Two separate groups of ten rats, with and without glucose infusion, were exercised with the same running protocol to ensure measurement of blood glucose. Plasma from tail vein blood samples (200 μL) was collected for the determination of glycaemia by means of the method coupling glucose oxydase and peroxydase (Glucose Enzymatique Color kit, Biotrol Diagnostic) with a spectrophotometric detection at 500 nm, identical to the method used for cerebral glucose measurement. Glucose levels were measured (i) before running, (ii) at the end running, (iii) at the end of recovery.

It was necessary to use different groups of rats in order to not disturb microdialysis experiment.

Data analysis

Glycaemia was expressed as the mean ± SE (μM) of concentration. The effect of exercise on these parameters was assessed by one-way analysis of variance (ANOVA). The effect of treatment (control vs. glucose infusion) was assessed by ANOVA.

Basal extracellular values of 5-HT [fmol (10 μL)$^{-1}$] and glucose [μmol (10 μL)$^{-1}$] were determined for each rat from the mean ± SE of concentrations in dialysate samples collected before exercise, not corrected for individual probe recoveries. During exercise and recovery, data were expressed as the mean ± SE of the percentage increase or decrease over basal concentrations in order do directly compare 5-HT and glucose changes. The effect of exercise on 5-HT and glucose was assessed by repeated measures ANOVA. Analysis also included a two-way ANOVA for repeated measure (treatment × time) to directly compare 5-HT and glucose changes with or without infusion.

Post-hoc analyses were performed using Newman–Keuls.

Significance was accepted at $P < 0.05$.

RESULTS

Effects of exercise

Glycaemia (n = 10). The mean value for glycaemia in rest conditions was 6.59 ± 0.14 mM. Exercise induced a slight but significant increase of glycaemia to

7.24 ± 0.23 mM ($P < 0.001$) at the end of running. Then, it decreased to reach 5.69 ± 0.32 mM ($P < 0.01$) at the end of recovery (Fig. 1).

Extracellular brain glucose ($n = 7$). Basal level of extracellular brain glucose was 193 ± 24 μM (10 μL)$^{-1}$. Exercise induced an early decrease to 91.7 ± 1.8% of baseline after 30 min of exercise ($P < 0.05$), then glucose increase to 121.1 ± 3.0% of baseline after 90 min of exercise ($P < 0.001$). Recovery induced a decrease to basal levels (Fig. 2).

Serotonin ($n = 7$). Basal level of extracellular hippocampal 5-HT was 16.6 ± 1.3 fmol (10 μL)$^{-1}$. Brain 5-HT shown an increase during exercise from the last 60 min, reaching 123.7 ± 6.4% of baseline at the end of exercise ($P < 0.001$) and 129.9 ± 7.0% after 30 min of recovery ($P < 0.001$). Then it decreased progressively to baseline after 2 h of recovery (Fig. 2).

Glucose and 5-HT presented similar changes during 90 min of exercise, but followed different pattern from 90 min of exercise to the end of recovery.

Effects of exercise plus glucose infusion

Glycaemia ($n = 10$). Basal level of glycaemia was 6.51 ± 0.13 mM. Exercise and glucose infusion induced a significant increase of glycaemia to 8.27 ± 0.28 mM ($P < 0.001$) at the end of running. Then, it decreased to 6.67 ± 0.2 mM at the end of recovery (Fig. 1).

Extracellular brain glucose ($n = 7$). Basal level of extracellular brain glucose was 188 ± 19 μM (10 μL)$^{-1}$. Exercise induced an early decrease to 88.1 ± 2.2% of baseline after 30 min of exercise ($P < 0.05$). A slight increase in baseline was observed at 60 min of exercise, then glucose decrease significantly during the end of exercise and recovery to reach 73.3 ± 4.6% of baseline after 90 min of recovery ($P < 0.001$). At the end of recovery, glucose was slightly increased but stayed significantly lower than baseline (79.6 ± 5.7%, $P < 0.001$) (Fig. 3).

Serotonin ($n = 7$). Basal level of extracellular brain 5-HT was 16.4 ± 0.8 fmol (10 μL)$^{-1}$. Brain 5-HT shown an increase from 60 min of exercise, reaching 134.5 ± 5.4% of baseline at the end of exercise ($P < 0.001$) and 141.0 ± 4.6% after 30 min of recovery ($P < 0.001$). During the first part of the recovery period 5-HT levels stayed maximal (142.0 ± 6.9%, after 90 min of recovery, $P < 0.001$), then decreased but stayed significantly higher than baseline at the end of recovery (123.8 ± 9.9%, $P < 0.05$) (Fig. 3). The 5-HT levels during infusion were significantly higher than the non-infused values during the last 90 min of recovery ($P < 0.001$).

A mirror profile between glucose and 5-HT was observed during all the glucose infusion.

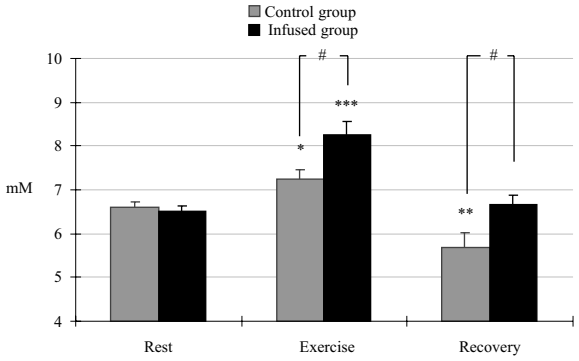

Figure 1 Effects of 2 h of exercise (25 m min^{-1}) on glucose concentrations in venous plasma without glucose infusion (*grey*; $n = 10$) and with glucose infusion during exercise and recovery (*black*; $n = 10$) (in concentration, compared to rest concentrations: $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$ and compared with each other: $\#P < 0.05$).

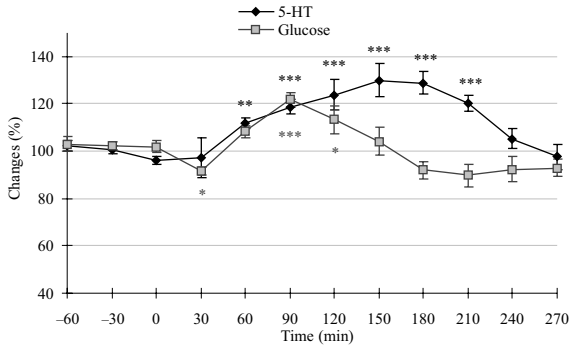

Figure 2 Effects of 2 h of exercise (25 m min^{-1}) on hippocampal glucose and serotonin levels ($n = 7$) (in %, compared with zero time point: $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$).

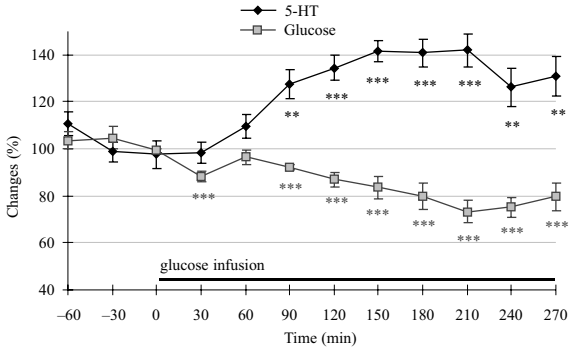

Figure 3 Effects of 2 h of exercise (25 m min^{-1}) on hippocampal glucose and serotonin levels, with glucose infusion (15%, 10 μL min^{-1}) during exercise and recovery ($n = 7$) (in %, compared with zero time point: $^{**}P < 0.01$; $^{***}P < 0.001$).

DISCUSSION

There are few previous studies where changes in glycaemia and brain glucose were related to neurotransmitter release: previous studies have reported that following a high carbohydrate diet there is an increase in brain serotonin synthesis (Fernstrom & Wurtman 1971) and release (Rouch *et al.* 1999). Another one has shown that cerebral glucose could be rapidly converted into brain amino acids, such as GABA and glutamate, and Vahabzadeh *et al.* (1995) have observed that changes in 5-HT are a mirror image of the glucose changes in hyperinsulinaemic situation. However, there is no previous study where changes in extracellular brain glucose concentration have been related to exercise nor to transmitter release when exercise occurred. This study is the first to directly compare glycaemia, brain glucose and 5-HT levels before, during and after an acute intensive exercise.

The glycaemia showed a slight increase which was induced by exercise. As animals were not fed just before experiment, it is probably because of peripheral regulations, such as glycogenolysis, which increase plasma glucose availability for muscles (Coyle *et al.* 1986). Then, blood glucose decreased and animals were hypoglycaemic after 2 h of recovery. This observation, previously described by numerous authors (Levine *et al.* 1924, Gibson & Edwards 1985), has been explained by the glucose utilization by muscles during exercise as main energetic substrate and by the glucose uptake into muscles and liver. When glucose infusion was performed, blood glucose levels were increased during all the experiments, inducing a higher increase after exercise and preventing a decrease under baseline at the end of recovery. Considering the important role of blood glucose levels on performance and fatigue, these results showed that our glucose supplementation would be sufficient to induce a beneficial peripheral effect during exercise and recovery.

In the exercise situation, cerebral glucose seemed to follow glycaemic variations – it increased during exercise, then decreased from 1 h 30 min of exercise to 1 h of recovery. This latter observation confirms previous studies about glucose transfer between blood to brain extracellular compartment, which showed that brain extracellular glucose levels mainly reflected glycaemic variations (Lund-Andersen 1979, Gruetter *et al.* 1998, Béquet *et al.* 2000). However, we observe some specific responses of extracellular brain glucose independent from glycaemic changes. Indeed, we did not observe a brain glucose decrease under baseline after recovery, in spite of hypoglycaemia. This result is consistent with a previously described phenomenon which evidenced a specific regulation of cerebral glucose metabolism after exercise (Béquet *et al.* 2000), implicating an enhanced glucose uptake by brain in recovery (Ide *et al.* 2000) and probably a glucose delivery to neurones by astrocytes. Indeed, exercise-induced peripheral changes are known to activate the brain glycogen metabolic cascade in astrocytes, increasing thereby the amount of extracellular glucose (Goldberg & O'Toole 1969, Magistretti 1988, Forsyth 1996). These data indicate that there is undoubtedly modifications of the brain glucose system during exercise which are not under the strict dependence of glycaemia.

The results obtained in the second situation, when glucose solution was infused, were far more surprising. As glycaemia was higher after infusion in the treated group, we expected higher extracellular glucose levels as well, as previously observed (Béquet *et al.* 2000). But on the contrary, the combination of both conditions, exercise and glucose infusion, inhibited the exercise-induced extracellular glucose increase in the first part of the exercise and thus, decreased brain glucose levels significantly under baseline during exercise and recovery. This phenomenon can hardly be explained because of the very complex regulation of glucose in brain. One probable hypothesis concern the role of insulin in glucose regulation. Indeed, a glucose supplementation is known to induce a high increase of blood insulin levels (Murray *et al.* 1989, MacLaren *et al.* 1999) and insulin is known to favour the glucose uptake in cellular structures in the peripheral system (Goodyear & Kahn 1998, Borghouts & Keizer 1999), but also in brain (Knudsen *et al.* 1999). Insulin is known to play an important role in brain glucose regulation, via the glycogen metabolism, especially during glucose supplementation (Hamai *et al.* 1999) and the insulin-sensitive transporter Glut4 is present in different brain structures brain (El Messari *et al.* 1998, Apelt *et al.* 1999). Thus, the increase of extracellular glucose may have been prevented by an enhanced entrance of glucose in brain intracellular compartment favoured by insulin, either in neurones for immediate consumption, either in glial cells for reserve. A second hypothesis concerns the glucocorticoids metabolism. Exercise induce a significant rise in glucocorticoids, which are strongly involved in cerebral glycogen catabolism in astrocytes (Goldberg & O'Toole 1969), and thus, leads to an increase in extracellular glucose content. Glucose supplementation is known to reduce the exercise-induced increase of glucocorticoids levels and it could decrease the amount of glucose released by astrocytes in extracellular compartment. Both hypothesis may partly explain the phenomenon, which implicates a regulation of glucose transfer between blood to CSF by astrocytes glycogen metabolism (Forsyth 1996).

The serotonergic response to exercise, as observed in the first situation, was consistent with our previously obtained results: hippocampal 5-HT began to

increase after 1 h of exercise and reached a maximum after 1 h of recovery; then it decreased to baseline at the end of recovery (Gomez-Merino *et al.* 2001). The increase in 5-HT levels, which is associated to central fatigue phenomena by numerous authors (Newsholme *et al.* 1987, Bailey *et al.* 1993a, b), seems to be related to the herein observed brain glucose variations. A relationship between the observed changes in brain glucose and 5-HT levels seems to occur during the experiment (i) glucose and 5-HT brain levels were increased during 90 min of exercise (ii) from 90 min of exercise to the end of recovery brain glucose decreased and 5-HT still increased. Moreover, when glucose was infused, the consequence was an enhanced increase of 5-HT levels, significant at the end of recovery, which prevented their return to baseline, and induced an earlier dissociation between brain glucose and 5-HT changes. Again, insulin might be implicated in these observations. On the one hand, carbohydrate diet increases plasma insulin, 5-HT synthesis (Fernström and Wurtman) and 5-HT release (Rouch *et al.* 1999). On the other hand, insulin infusion increases 5-HT synthesis (Fernström and Wurtman) and 5-HT release in hippocampus (Vahabzadeh *et al.* 1995). Thus our glucose infusion may have induced an increase of plasma insulin, which is known to favour the uptake of tryptophan, the 5-HT precursor, in brain and thus to induce 5-HT synthesis. But Vahabzadeh *et al.* observed an immediate increase of brain extracellular 5-HT after the insulin injection and it seems most improbable that the involvement of 5-HT synthesis pattern is responsible for such a rapid phenomenon. Moreover, if Vahabzadeh *et al.* showed an increase of extracellular 5-HT levels in hippocampus after insulin infusion, Orosco & Nicola (1994) showed a decrease in hypothalamus. Thus, it is difficult to conclude on the role of insulin, as its relationship with 5-HT is complex and probably area-dependent.

In their study, Vahabzadeh *et al.* have shown that the injection of insulin induced a decrease of hippocampal glucose levels. More than that, they have shown that a subsequent glucose infusion increased hippocampal glucose levels and was able to decrease 5-HT levels to baseline. During all their experiment, 5-HT was a mirror image of the extracellular glucose changes. In our physical exercise + infusion condition, we observed the same mirror image: extracellular brain glucose was significantly decreased under baseline and 5-HT was increased compared with the non-infused situation. Our experiment, according to Vahabzadeh results, suggest a direct influence of extracellular brain glucose on the 5-HT system. Glucose levels in extracellular compartment in brain seems to be the reflection of a complex balance between peripheral and central metabolisms, the influence of the peripheral metabolism depending on glycaemia as well as insulinaemia. These latter mechanisms could interact to regulate extracellular glucose levels and consequently would influence the 5-HT system.

To our knowledge, no description of a possible regulation of the 5-HT metabolism directly by brain glucose have previously been made. It seems possible that the action of glucose on 5-HT implicate an intermediate. The 5-HT release is under the influence of numerous central mechanisms and numerous neurotransmitters as GABA, glutamate, dopamine may modulate it via 5-HT heteroreceptors on the serotonergic neurone. The GABA could be particularly interesting as it is a inhibitor of the 5-HT release and as its synthesis is directly dependant to brain glucose content. Thus, the glucose lowered levels could have limited GABA synthesis and release, and so prevented the inhibition of 5-HT release. A second hypothesis could be a direct action of extracellular glucose on serotonergic neurones, either via a glucose-sensing phenomenon (Maekawa *et al.* 2000), either by a direct action on 5-HT heteroreceptors, but such a phenomenon has not been described. At last, Orosco *et al.* (2000) have shown an increase of extracellular 5-HT in hypothalamus 45 min after an insulin injection in this brain area and Gerozissis *et al.* (1999) have shown that a glucose supplementation could increase brain insulin levels in some brain areas. It suggests that brain insulin could also play a role in the complex relation between extracellular glucose and 5-HT during exercise, with or without glucose infusion.

Our results, according to the other studies, favour the hypothesis of a direct influence of extracellular glucose availability on 5-HT metabolism. It seem possible that, on one hand, increased insulin induces 5-HT synthesis via TRP availability as previously described (Chaouloff 1997) but, on the other hand, that insulin increases 5-HT release by an induced brain glucose lowered levels. Taking into account the implication of brain 5-HT in central fatigue, our data, showing an increase of 5-HT after exercise, implicate that glucose infusion would increase the sense of fatigue during recovery. This hypothesis suggest that if glucose supplementation, before and during exercise, undoubtedly increase performance because of its peripheral positive action, it would have a negative impact on the quality of recovery after the end of the exercise.

We are particularly indebted to Ms D. Bonneau, from the IMASSA's Department of Chemistry, for her help.

REFERENCES

Apelt, J., Mehlhorn, G. & Schlieb, R. 1999. Insulin-sensitive GLUT4 glucose transporters are colocalized with

GLUT3-expressing cells and demonstrate a chemical distinct neuron-specific localization in rat brain. *J Neurosci Res* **57,** 693–705.

Bailey, S.P., Davis, J.M. & Ahlborn, E.N. 1993a. Neuroendocrine and substrate responses to altered brain 5-HT activity during prolonged exercise to fatigue. *J Appl Physiol* **74,** 3006–3012.

Bailey, S.P., Davis, J.M. & Ahlborn, E.N. 1993b. Serotonergic agonists and antagonists affect endurance performance in the rat. *Int J Sports Med* **14,** 330–333.

Béquet, F., Pérès, M., Gomez-Merino, D. *et al.* 2000. Simultaneous NMR-microdialysis study of brain glucose metabolism in relation to fasting or exercise in the rat. *J Appl Physiol* **88,** 1949–1954.

Bergström, J. & Hultman, E. 1967. A study of glycogen metabolism during exercise in man. *Scand J Clin Lab Invest* **19,** 218–228.

Bigland-Ritchie, B., Furbush, F. & Woods, J.J. 1986. Fatigue of intermittent submaximal voluntary contractions: central and peripheral factors. *J Appl Physiol* **61,** 421–429.

Borghouts, L.B. & Keizer, H.A. 1999. Exercise an insulin sensitivity: a review. *Int J Sports Med* **20,** 1–12.

Chaouloff, F. 1997. Effect of physical exercise on central serotoninergic systems. *Med Sci Sports Exerc* **29,** 58–62.

Chaouloff, F., Elghozi, J.L., Guezennec, C.Y. & Laude, D. 1985. Effects of conditioned running on plasma, liver and brain tryptophan and on brain 5-HT metabolism of the rat. *Br J Pharmacol* **86,** 33–41.

Coyle, E.F., Coggan, A.R., Hemmert, M.K. & Ivy, J.L. 1986. Muscle glycogen utilization during prolonged strenuous exercise when fed carbohydrates. *J Appl Physiol* **61,** 165–172.

El Messari, S., Leloup, C., Guignon, M., Brisorgueil, M.J., Penicaud, L. & Arluison, M. 1998. Immunocytochemical localization of the insulin-responsive glucose transporter 4 (Glut4) in the rat central nervous system. *J Comp Neurol* **399,** 492–512.

Fernstrom, J.D. & Wurtman, R.J. 1971. Brain serotonin content: increase following ingestion of carbohydrate diet. *Science* **174,** 1923–1925.

Forsyth, R.J. 1996. Astrocytes and the delivery of glucose from plasma to neuron. *Neurochem Int* **28,** 231–241.

Gerozissis, K., Rouch, C., Nicolaidis, S. & Orosco, M. 1999. Brain insulin response to feeding in the rat is macronutrient and area specific. *Physiol Behav* **66,** 271–275.

Gibson, H. & Edwards, R.H.T. 1985. Muscular exercise and fatigue. *Sports Med* **2,** 120–132.

Goldberg, N.D. & O'Toole, A.G. 1969. The properties of glycogen synthetase and regulation of glycogen biosynthesis in rat brain. *J Biol Chem* **244,** 3053–3061.

Gomez-Merino, D., Béquet, F., Berthelot, M., Chennaoui, M. & Guezennec, C.Y. 2001. Site-dependent effects of an acute intensive exercise on extracellular 5-HT and 5-HIAA levels in rat brain. *Neurosci Lett* **301**(2)**,** 143–146.

Goodyear, L.J. & Kahn, B.B. 1998. Exercise, glucose transport, and insulin sensitivity. *Annu Rev Med* **49,** 235–261.

Gruetter, R., Ugurbil, K. & Seaquist, E.R. 1998. Steady-state cerebral glucose concentrations and transport in the human brain. *J Neurochem* **70,** 397–408.

Hamai, M., Minokoshi, Y. & Shimazu, T. 1999. L-Glutamate and insulin enhance glycogen synthesis in cultured astrocytes from the rat brain through different intracellular mechanisms. *J Neurochem* **73,** 400–407.

Ide, K., Schmalbruch, I.K., Quistorff, B., Horn, A. & Secher, N.H. 2000. Lactate, glucose and O_2 uptake in human brain during recovery from maximal exercise. *J Physiol* **522,** 159–164.

Knudsen, G.M., Hasselbalch, S.G., Hertz, M.M. & Paulson, O.B. 1999. High dose insulin does not increase glucose transfer across the blood–brain barrier in humans: a re-evaluation. *Eur J Clin Invest* **29,** 687–691.

Koslowski, S., Brzezinska, Z., Nazar, K. & Turlejska, E. 1981. Carbohydrate availability for the brain and muscle as a factor modifying sympathetic activity during exercise in dogs. In: J. Poortmans & G. Niset (eds) *Biochemistry of Exercise,* chapt IV B, pp. 54–62. University of Park Press, Baltimore.

Levine, S.A., Gordon, B. & Derick, C.L. 1924. Some changes in the chemical constituents of the blood following a marathon race. *JAMA* **82,** 1778–1779.

Lund-Andersen, H. 1979. Transport of glucose from blood to brain. *Physiol Rev* **59,** 305–351.

MacLaren, D.P., Reilly, T., Campbell, I.T. & Hopkin, C. 1999. Hormonal and metabolic responses to maintained hyperglycaemia during prolonged exercise. *J Appl Physiol* **87,** 124–131.

Maekawa, F., Toyoda, Y., Torii, N. *et al.* 2000. Localization of glucokinase-like immunoreactivity in the rat lower brain stem: for possible location of brain glucose-sensing mechanisms. *Endocrinology* **141,** 375–384.

Magistretti, P.J. 1988. Regulation of glycogenolysis by neurotransmitters in the central nervous system. *Diabete Metab* **14,** 237–246.

Murray, R., Paul, G.L., Seifter, G., Eddy, D.E. & Halaby, G.A. 1989. The effect of glucose, fructose, and sucrose ingestion during exercise. *Med Sci Sports Exerc* **21,** 275–282.

Neufer, P.D., Costill, D.L. & Flynn, M.G. 1987. Improvements in exercise performance: effects of carbohydrate feeding and diet. *J Appl Physiol* **62,** 983–988.

Newsholme, E.A., Acworth, I. & Blomstrand, E. 1987. Amino-Acids, brain neurotransmitters and a functional link between muscle and brain that is important in sustained exercise. In: G. Benzi (ed.) *Advances in Biochemistry*, pp. 127–133. John Libbey Eurotext Ltd, London.

Nicolaidis, S., Rowland, N., Meile, M.J., Marfaing-Jallat, P. & Pesez, A. 1974. A flexible technique for long-term infusions in unrestrained rats. *Pharmacol Biochem Behav* **2,** 131–136.

Orosco, M. & Nicola, S. 1994. Insulin and glucose-induced changes in feeding and medial hypothalamic monoamines revealed by microdialysis in rats. *Brain Res Bull* **33,** 289–297.

Orosco, M., Rouch, C. & Gerozissis, K. 2000. Activation of hypothalamic insulin by serotonin is the primary event of the insulin–serotonin interaction involved in the control of feeding. *Brain Res* **872,** 64–70.

Paxinos, G. & Watson, C. 1982. *The Rat Brain in Stereotaxic Coordinates.* Academic Press, New York.

Pennington, A.J. & Pentreath, V.W. 1987. Transmitter-induced glycogenolysis and gluconeogenesis in leech segmental ganglia. *J Physiol* **82,** 218–228.

Poblete, J.C. & Azmitia, E.C. 1995. Activation of glycogen phosphorylase by serotonin and 3,4-methylendioxy metyhamphetamine in astroglial-rich primary cultures: involvement of the 5-HT2A receptor. *Brain Res* **680,** 9–15.

Rouch, C., Nicolaidis, S. & Orosco, M. 1999. Determination, using microdialysis, of hypothalamic serotonin variations in response to different macronutrients. *Physiol Behav* **65,** 653–657.

Saltin, B. & Karlsson, J. 1971. Muscle glycogen utilization during work of different intensities. In: B. Pernow & B. Saltin (eds) *Advances in Experimental Medicine and Biology*, vol. 11, pp. 289–299. Plenum, New York.

Vahabzadeh, A., Boutelle, M.G. & Fillenz, M. 1995. Effects of changes in rat brain glucose on serotonergic and noradrenergic neurons. *Eur J Neurosci* **7,** 175–179.

Wong, K.L. & Tyce, G.M. 1983. Glucose and amino acid metabolism in rat brain during sustained hypoglycaemia. *Neurochem Res* **8,** 401–415.