Brain metabolism of glucose and lactate was analyzed by ex vivo NMR spectroscopy in rats presenting different cerebral activities induced after the administration of pentobarbital, α-chloralose, or morphine. The animals were infused with a solution of either [1-13C]glucose plus lactate or glucose plus [3-13C]lactate for 20 min. Brain metabolite contents and enrichments were determined from analyses of brain tissue perchloric acid extracts according to their post-mortem evolution kinetics. When amino acid enrichments were compared, both the brain metabolic activity and the contribution of blood glucose to brain metabolism were linked with cerebral activity. The data also indicated the production in the brain of lactate from glycolysis in a compartment other than the neurons, presumably the astrocytes, and its subsequent oxidative metabolism in neurons. Therefore, a brain electrical activity-dependent increase in the relative contribution of blood glucose to brain metabolism occurred via the increase in the metabolism of lactate generated from brain glycolysis at the expense of that of blood lactate. This result strengthens the hypothesis that brain lactate is involved in the coupling between neuronal activation and metabolism.

In the last decade, the idea of the involvement of lactate in the coupling between neuronal activation and energy metabolism has arisen from the results of various experimental investigations. Briefly, in vitro studies have evidenced lactate release from astrocytes after glycolysis stimulation by glutamate uptake (1) and, in the particular case of the mammalian retina, the use of lactate from Müller cells as an energy substrate for photoreceptors (3). In vivo studies on brain have demonstrated the uncoupling of oxygen and glucose utilization (4) and the release of lactate into the extracellular fluid in response to neuronal activation (5, 6). On the other hand, brain lactate has been demonstrated to efficiently protect neurons against delayed ischemic damage (7). The metabolic fate of exogenous lactate in whole brain has been investigated by ex vivo NMR spectroscopy. In this way, it has been demonstrated that blood lactate enters the brain and is more specifically metabolized in neurons (8–10). The proposed astrocyte-neuron lactate shuttle hypothesis (ANLSH) (1) as the coupling model between neuronal activity and energy metabolism requires the involvement of different components, the occurrence and localization of which have been investigated analytically at the molecular level, i.e. glutamate transporter and Na⁺,K⁺-ATPase (11), lactate dehydrogenase isoenzymes (12), and monocarboxylate transporters (13, 14). Although brain lactate production and lactate use by neurons as an energy source are widely admitted, the relevance of the coupling model, particularly the neuronal utilization of glia-produced lactate, is a topical issue (15, 16).

Brain lactate mostly derives from glycolysis. Therefore, the comparative analysis of the contribution of brain lactate and glucose to the oxidative metabolism in brain cells cannot be achieved in a simple manner. Indeed, using labeled glucose alone is of no use, because labeled lactate will be formed and there will be no way to further discriminate between the metabolism of these two compounds. On the other hand, using labeled lactate alone gives information on the metabolism of the exogenous lactate but not on the metabolism of brain endogenous lactate. To investigate in vivo the relevance of the ANLS hypothesis, we therefore used an alternative approach in which brain lactate metabolism was investigated by analyzing the competition between the lactate endogenous to brain and the exogenous lactate intravenously administrated to the animals through the infusion of a solution of either [1-13C]glucose plus lactate or glucose with [3-13C]lactate. Thus, using rats treated with various drugs leading to different levels of cerebral activity, we recently demonstrated from the analysis of alanine, lactate, and acetyl-CoA labeling that astrocytic glycolysis was the main source of the endogenous brain lactate and that the contribution of blood glucose relative to the contribution of blood lactate to the neuronal metabolism was linked to brain activity (17). Using the same experimental approach, in the present study we analyzed the amino acid enrichments reached under the different conditions. A crucial requirement was the determination of brain metabolite content and enrichment in brain tissue, keeping in mind that there was a risk of bias due to the possible evolution of the metabolite content in the time interval between blood circulation arrest and tissue freezing. This was particularly important for glucose and lactate because of anaerobic glycolysis. To control this process, we determined the time dependence of brain glucose, lactate, and amino acid content and 13C-enrichments after blood circulation arrest. Thus, comparing alanine and lactate enrichments under the different conditions confirmed the compartmentation of their metabolism. Moreover, from the enrichment of the amino acids, we found that the contribution to their metabolism of blood glucose relative to that of blood lactate increased with brain activation. This increase is discussed in terms of a higher production and metabolic consumption of brain glycolytic lactate with brain activation.
Lactate in Brain Activity-Metabolism Coupling

EXPERIMENTAL PROCEDURES

Anesthetics—The experimental protocols used in this study were approved by the appropriate institutional review committees and met the guidelines of the appropriate governmental agency (authorization number 7368). Female Wistar rats (200 g) were fasted overnight. They received intraperitoneal injection of the following anesthetics and analgesics: 90 mg/kg sodium pentobarbital, 80 mg/kg α-chloralose, or 50 mg/kg morphine sulfate. To avoid hypothermia, they were kept under a glow lamp during the experiments.

Labeled Substrate Infusion—About 15 min after drug administration, the rats were infused in a tail vein for 20 min with a saline solution containing either 750 mM 99% enriched [1-13C]glucose and 500 mM lactate or 500 mM glucose and 500 mM 99% enriched [3-13C]lactate. The infusion flow was monitored to follow a time-decreasing exponential as described previously (8). A few minutes before the end of infusion, a longitudinal incision was made on the scalp of the animals, and its two parts were drawn aside to make the cranium apparent. For the animals under α-chloralose or morphine, this incision was made using lidocaine as a local anesthetic. The rat head was then secured by pinning the scalp on a holder adapted to its shape. At the end of infusion, it was immediately split in two by a hammer stroke onto a sharp knife maintained on the cranium on the longitudinal axis. The two cerebral hemispheres were then easily removed and dipped into liquid nitrogen.

To obtain relevant data on both the brain metabolite contents and the enrichments at the end of infusion, the post-mortem effects occurring between blood circulation arrest and tissue freezing were determined as follows. Considering the instant when the cranium was split in two and the cerebral hemispheres were removed, the cerebral hemispheres were dipped into liquid nitrogen either immediately, i.e. at a measured time ranging from 6 to 12 s for the first removed hemisphere, or after a given delay of 30, 60, 90, or 120 s. After metabolite extraction, the time evolution of the glucose, the lactate, the amino acid contents, and the enrichments were analyzed. A minimal number of three animals were used for each time.

During the last minute of infusion, a blood sample (~400 μl) was collected by cardiac puncture. It was immediately mixed with 15 μl of perchloric acid (9 n) and centrifuged. The supernatant was removed and adjusted to pH 4. A 50-μl aliquot was taken for glucose and lactate determination, and the remainder was lyophilized. The resulting powder was dissolved in 500 μl of D2O for 1H-NMR analysis.

The animals treated with morphine sulfate were immobilized before starting the infusion. A foam rubber cylinder (size, 20 cm; interior diameter, 4-cm) was used as a restrainer. A longitudinal (dorsal) opening was made on a side of the cylinder to put the animal inside, and four holes (2 × 1 cm) were made on the opposite side to allow the paws of the animals to stand outside. Five longitudinal windows (7 × 0.5 cm) were opened between these holes both to provide to the cylinder part that was in contact with the animal thorax and abdomen the flexibility required for breathing movements and to give access for cardiac puncture. After the animal was correctly positioned inside the cylinder, the longitudinal opening was closed with adhesive tape. During infusion, the cylinder was maintained so that the paws did not come into contact with the working surface.

Control animals perfused under the same conditions with unenriched lactate and glucose were used to determine blood PaO2, PaCO2, and pH. Five minutes before the end of infusion, the carotid artery of the rats was exposed to collect a 300–500-μl blood sample. Blood gases and pH were then immediately determined using a Radiometer ABL5 analyzer.

Metabolite Extraction—The frozen cerebral tissue was pulverized under liquid nitrogen with a pestle and mortar. A volume of 5 ml of 0.9% perchloric acid was then added drop by drop at the nitrogen surface, and the frozen droplets were immediately pulverized. The mixture was transferred to a Dounce homogenizer and homogenized at 4 °C after thawing. After centrifugation (10,000 × g for 20 min), the supernatant was neutralized with KOH, centrifuged to eliminate perchlorate salts, and freeze-dried. The pellet was dissolved in 0.2 M Tris-base 2% sodium dodecyl sulfate for protein determination.

Glucose, Lactate, and Protein Determination—Glucose and lactate contents in blood and brain extracts were determined by enzymatic assays using the kits from Sigma. Protein contents in brain tissue were determined as described (18).

NMR Spectroscopy—For NMR spectroscopy, each sample was dissolved in 500 μl D2O. Spectra were obtained with a Bruker Avance 500 narrow bore spectrometer equipped with a 5-mm broad band probe. Measurements were performed at 30 °C. Proton-decoupled 13C-NMR spectra of perchloric acid extracts were acquired under the conditions of a 5.1-μs pulse (52° flip angle), 1.18-s acquisition time, 0.1-s relaxation delay, 22,150 Hz sweep width, 64K memory, gated proton composite pulse decoupling, and D2O lock. The number of scans was 50,000. 1H-NMR spectra were acquired using a 1.3-μs pulse (90° flip angle), 3.27-s acquisition time, 8.5-s relaxation delay, 5,000 Hz sweep width, and 32K memory size. The residual water signal was suppressed by homonuclear pre-saturation. The number of scans was 32. 1H-observed/13C-edited spectra were obtained as described previously (8). The acquisition sequences involved a proton spin echo with calibrated 90° and 180° pulses separated by a 3.94-ms delay corresponding to the 0.5 JCH value for the 1H-13C scalar coupling in amino acid -CH2- and -CH3 (JCH = 126–132 Hz) either with or without 13C spin inversion with a 90, 120, 120, and 90° composite pulse (8.75 μs for 90° flip angle). Other parameters were a 3.28-s acquisition time, 8.5-s relaxation delay, 5,000 Hz sweep width, and 32K memory size. Measurements were conducted under carbon decoupling and water proton presaturation. The free induction decays corresponding to the two types of data were alternatively acquired as three blocks of 64 scans. Proton-decoupled 31P-NMR spectra were acquired using a 8.5-μs pulse (90° flip angle), 0.81-s acquisition time, 10,080 Hz sweep width, 0.5-s relaxation delay, 32K memory size, and D2O lock. Spectra resulted from 10,000 scans. Before the 1P-NMR analysis, a 50-μl aliquot of EDTA (100 mM, pH 7) was added to the sample in order to form a complex of the divalent cations.

Statistical Analysis—Data are represented as the mean ± S.D. with n ≥ 3. The data were analyzed by analysis of variance followed by Bonferroni's test with p < 0.05 using the Statgraphics package software.
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Table I
Blood glucose, lactate, and alanine concentrations and enrichments after a 20-min infusion of either [1-13C]glucose plus lactate or glucose plus [3-13C]lactate in rats treated with pentobarbital, α-chloralose, or morphine

| Perfusate | Concentration (mM) or enrichment (%) | Pentobarbital | α-Chloralose | Morphine |
|-----------|--------------------------------------|---------------|--------------|----------|
| [1-13C]glucose + lactate and Glucose + [3-13C]lactate | [Glc] (mM) 18.3 ± 2.4 (n = 22) [Lac] (mM) 4.0 ± 0.6 (n = 21) [Ala] (mM) 0.34 ± 0.10 (n = 15) | 16.0 ± 1.5* (n = 17) 3.1 ± 0.8* (n = 16) 0.30 ± 0.07* (n = 12) | 18.8 ± 2.6 (n = 9) 7.0 ± 1.6* (n = 12) 0.58 ± 0.15* (n = 12) |
| [1-13C]glucose + lactate | [Glc C1] (%) 69.9 ± 5.0 [Lac C3] (%) 3.2 ± 0.8 [Ala C3] (%) 2.8 ± 0.4 | 71.6 ± 5.5 3.4 ± 0.3* 3.0 ± 1.0 | 72.4 ± 2.5 7.3 ± 1.6* 3.7 ± 1.1 |
| Glucose + [3-13C]lactate | [Glc C1] (%) 2.3 ± 1.5 [Lac C3] (%) 47.5 ± 2.6 [Ala C3] (%) 34.5 ± 4.0 | 3.1 ± 0.3 50.2 ± 3.4* 39.7 ± 9.5* | 3.3 ± 1.2 30.3 ± 2.2* 22.0 ± 2.4* |

* Significant difference between α-chloralose and morphine.

RESULTS

Physiological Status of the Infused Rats—As reported previously, an electroencephalogram recording indicated that the rat cerebral activity was strongly depressed under pentobarbital anesthesia, whereas a slight increase in spectral power in the 1–20 Hz frequency band was noted under both α-chloralose and morphine as a consequence of a large power increase in the δ band, whereas α-chloralose induced a slight power decrease in the θ band (17). At the end of infusion, PaO₂ was 74 ± 2 and PaCO₂ was 53 ± 1, or 82 ± 2 and PaCO₂ was 53 ± 2, 51 ± 1, or 40 ± 1 under pentobarbital, α-chloralose, or morphine anesthesia, respectively. The pH was 7.37 ± 0.03 with all three drugs.

Concentration and Specific Enrichment of Glucose, Lactate, and Alanine in the Blood and Brain of the Infused Rats—The rats were simultaneously infused with glucose and lactate to compare brain metabolite enrichments from [3-13C]lactate or [1-13C]glucose under the same metabolic conditions. As shown previously, infusing both substrates made it possible to reach higher blood concentrations than after the infusion of only one of them and minimized the consequences of hepatic gluconeogenesis on their labeling (8). After a 20-min infusion, glucose concentration was slightly lower under α-chloralose than under pentobarbital or morphine anesthesia, whereas lactate concentration was ~2-fold higher under morphine than under pentobarbital or α-chloralose (Table I). Glucose enrichments were the same under the three drugs as well as after [1-13C]glucose and [3-13C]lactate infusion (in this latter case, glucose enrichment reflected gluconeogenesis activity). Lactate enrichments were the same under pentobarbital and α-chloralose anesthesia; the enrichment value (~50% with the infusion of [3-13C]lactate) revealed an important isotopic dilution by glycolytic lactate. The glycolytic activity was even higher under morphine, leading to enrichment values of only 30% with [3-13C]lactate infusion and >7% with [1-13C]glucose infusion. The blood alanine content corresponded to around one-twelth of the lactate content with the three drugs. Alanine enrichment was similar to that of lactate with [1-13C]glucose plus lactate infusion except under morphine, where the amino acid enrichment was half that of lactate. Under the infusion of glucose plus [3-13C]lactate, alanine enrichment was 20–25% less than that of lactate.

The contents and enrichments of brain glucose, lactate, and alanine at the end of infusion are reported in Table II. These data were determined by analyzing the time-dependent evolutions of brain glucose, lactate, and alanine after blood circulation arrest (Figs. 1, 2, and 3). In the post-mortem phase, the rate of lactate content increase corresponded roughly to twice that of glucose consumption, indicating that the latter was essentially degraded into lactate. Comitantly, the alanine content increase was much slower. Lactate C3-specific enrichment increased with time under [1-13C]glucose plus lactate infusion, whereas it decreased under glucose plus [3-13C]lactate infusion as a result of the glycolytic production of either labeled or unlabeled lactate, respectively. Alanine enrichment changes were in the same direction but much less intense. The post-mortem metabolic activity was also characterized by changes in energy metabolites. From 31P-NMR spectra, it was found that the NTP/NPD ratio drastically dropped from 3.2–4 after a few seconds to 1 at 2 min. This was accompanied by a >90% decrease in phosphocreatine content. On the other hand, 13C- and 31P-NMR spectra revealed the presence of both glycero-3-phosphate and dihydroxyacetone phosphate, the contents of which increased at least 2-fold in the post-mortem phase. Spectra in Fig. 4 illustrate the increase in these compounds and in lactate and alanine. As reported in Table II, the glucose contents at time zero were significantly different under the three drugs, with the highest and lowest values found under pentobarbital and α-chloralose, respectively. The lactate content was higher under morphine than under pentobarbital, whereas the content in alanine was the same under the three drugs. Brain glucose enrichments were the same under the three drugs and identical to those of blood glucose. From pentobarbital to morphine, lactate and alanine enrichments either increased or decreased with the infusion of either [1-13C]glucose plus lactate or glucose plus [3-13C]lactate, respectively.

Amino Acid Contents and Enrichments in the Infused Rat Brains—For the amino acids other than alanine, no significant change in content or enrichment was evidenced on the 0–2 min post-mortem time scale (data not shown). The amino acid contents were the same under the different drugs, except for aspartate, whose content was lower under morphine (data not shown). Ala C3 was the most enriched amino acid carbon, both compared with those of the other amino acids, as was already observed (19). Indeed, alanine is directly connected with pyruvate through aminotransferase activity, whereas the metabolic pathways leading to the other amino acids involve multisteps (the carbon in the pyruvate C3 position gives the C2 of acetyl-CoA, which becomes the C4 of 2-oxoglutarate and, further, the C3 or C2 of oxaloacetate in the first tricarboxylic acid cycle turn; from these intermediates, the carbon may be recovered either at glutamate C4, glutamine C4, GABA C2, or aspartate C2 or C3). Moreover, because of its small pool size, alanine turnover is faster than that of the other amino acids.

For all of the amino acids there was an enrichment increase from pentobarbital to α-chloralose and morphine when the labeled substrate was [1-13C]glucose. On the contrary, the enrichments were either the same or slightly decreased when the labeled substrate was [3-13C]lactate. However, the ratio be-
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The data were obtained after a 20-min infusion of either $[1^{-13}C]$glucose plus lactate or $[3^{-13}C]$lactate plus glucose in rats treated with either pentobarbital, $\alpha$-chloralose, or morphine. Data were analyzed as described under “Experimental Procedures.” $k_i$ is the rate of glycolysis and $k_2$ is the rate of lactate production. $\text{Glc}_i$, $\text{Ala}_i$, and $\text{Lac}_i$ and $\text{Glc}_0$, $\text{Ala}_0$, and $\text{Lac}_0$ are the brain glucose, alanine, and lactate contents and enrichments at the end of infusion, respectively. Metabolite contents are expressed as nmol/mg protein. The values for the $k_i$ and $k_2$ rates and the metabolite contents were obtained from data corresponding to the two infusion conditions.

### Table II

| Labeled substrate | Labeled substrate | Labeled substrate |
|-------------------|-------------------|-------------------|
| $[1^{-13}C]\text{Glc}$ | $[3^{-13}C]\text{Glc}$ | $[1^{-13}C]\text{Lac}$ | $[3^{-13}C]\text{Lac}$ |
| % | % | % | % |
| $\text{Glc C1}$ | $\text{Glc C2}$ | $\text{Glc C3}$ | $\text{Glc C4}$ |
| 72.5 ± 2.2 | 2.8 ± 1.2 | 71.7 ± 4.0 | 3.5 ± 1.2 |
| $\text{Lac C1}$ | $\text{Lac C2}$ | $\text{Lac C3}$ | $\text{Lac C4}$ |
| 11.2 ± 2.8 | 19.7 ± 0.4 | 16.2 ± 1.7 | 11.2 ± 0.7 |
| $\text{Ala C1}$ | $\text{Ala C2}$ | $\text{Ala C3}$ | $\text{Ala C4}$ |
| 10.3 ± 0.3 | 24.5 ± 0.9 | 17.9 ± 0.6 | 19.6 ± 0.9 |

$^a$ Significant difference between pentobarbital and $\alpha$-chloralose.
$^b$ Significant difference between $\alpha$-chloralose and morphine.
$^c$ Significant difference between $\text{Glc}_i$ and $\text{Ala}_i$.

### Discussion

**Evolution of Brain Metabolites during the Ischemic Episode following Brain Tissue Removal**—The procedure that we used to remove the cerebral tissue of the anesthetized rats is particularly simple and easy to perform. After instantaneous cleavage of the cranium, which gives access to the two brain hemispheres, excising and freezing the tissue in liquid nitrogen takes <10 s. The procedure avoids the difficult dissection of the frozen tissue obtained after the immersion in liquid nitrogen of the decapitated rat head or after brain funnel freezing. The NTP/NDP ratio that we obtained was similar to the ATP/ADP ratio reported by using the brain blowing method when the turnover time of these metabolites is within a few seconds (21). Relevant data on $in vivo$ concentrations of metabolites prone to evolve post mortem were thus determined from a kinetic study. An intense and rapid increase in lactate content was observed during the ischemic episode resulting from brain tissue removal, thus demonstrating the recruitment of glycolysis to sustain the best cell energy level. The glucose degradation rate appeared proportional to the glucose content (data in Table II, the ratio $k_2/[\text{Glc}_i] = 5.9 \pm 0.3 \times 10^{-3} \text{ s}^{-1}$ was constant), thus suggesting that the degradation was not a simple Michaelian process. The glucose degradation rate amounted to a 1.5–2.3-μmol glucose unit per gram per minute (data in Table II assuming 100 mg of protein per gram of tissue), i.e. a value greater than the $in vivo$ glucose consumption (0.08–0.5 μmol/g/min) (22). However, based on ATP production from oxidative or anaerobic glucose consumption (36 or 2 ATP/glucose), the anaerobic glycolysis was insufficient to maintain the cell energy status, thus inducing the fall in phosphocreatine and ATP contents. Lactate was the major glycolytic product. In addition to lactate synthesis, which regenerated the NAD$^+$ necessary to maintain the glycolytic activity, the alanine content was increased via aminotransferase activity, probably as the consequence of an increased pyruvate concentration. A fraction of the glycolytic flux was also devoted to glycerol 3-phosphate synthesis through glycerol phosphate dehydrogenase, which also regenerated NAD$^+$. As compared with lactate, glycerol 3-phosphate accumulation has been described as a criterion for severe hypoxia (23). An increase in dihydroxyacetone phosphate was also observed that revealed glycolysis activation through that of phosphofructokinase (24), the cell metabolism being focused on maintaining the cell redox and energy status under the ischemic insult. Despite the collapse of oxidative metabolism, the contents in amino acids other than alanine were not affected in the short term of the ischemic episode.

**Glucose and Lactate Concentrations and Enrichments in Blood and Brain**—Hyperglycemia was obtained during the infusions. However, blood glucose was slightly lower under $\alpha$-chloralose than under pentobarbital or morphine. Under $\alpha$-chloralose and pentobarbital, brain glucose was correlated to blood glucose in very good agreement with the linear dependences reported by Choi et al. (25) and explained by the reversible Michaelis-Menten model (26). The higher glucose level under pentobarbital anesthesia was the consequence of the depressed metabolic activity with that drug. Indeed, although pentobarbital reduces the transfer into the brain of blood glucose (27) through a direct inhibitory effect upon the transporters (28), the high brain glucose level with that drug was mostly the consequence of the drop in cerebral glucose consumption (29). Under morphine, the ratio brain glucose/blood glucose was the same as under $\alpha$-chloralose anesthesia (0.26 and 0.27, respectively, assuming 100 mg of protein per milliliter of tissue), suggesting that the linear dependences under these two drugs were very close.

Blood lactate concentrations were ~50% higher in the rats
under morphine anesthesia than in those under pentobarbital or α-chloralose. This was likely due to the generation of endogenous glycolytic lactate, because lactate C3 enrichment after [3-13C]lactate plus glucose infusion was 40% lower in the morphine-treated than in the pentobarbital- or α-chloralose-treated rats. As a consequence, on the basis of a near linear concentration-dependent brain lactate influx through both a saturable and a non-saturable transport component (30), the brain lactate levels for the rats under morphine were also higher (Table II). Conversely, in these rats, the high blood lactate concentration might have hindered the efflux of lactate generated in the brain with time.

Glycolytic activity was higher under morphine than under pentobarbital or α-chloralose, as revealed both by the lower and higher lactate enrichment with glucose plus [3-13C]lactate and [1-13C]glucose plus lactate infusion, respectively (Table I). Alanine was also found in the blood at a concentration corresponding to around one-twelfth that of lactate. Its enrichment was slightly less than that of lactate, suggesting that the occurrences of these two compounds were connected, probably through liver metabolism. It was controlled that under the conditions of the present study the contribution of blood alanine to brain metabolism was negligible (data not shown).

Involvement of Brain Lactate in the Neuronal Metabolism—With [1-13C]glucose plus lactate infusion, brain lactate enrichment was 11, 16, or 22% (Table II), whereas blood lactate enrichment was only 3.2, 3.4, or 7.3% (Table I) under pentobarbital, α-chloralose, and morphine anesthesia, respectively, thus demonstrating the occurrence of an endogenous brain lactate production from [1-13C]glucose. Indeed, as blood and
brain glucose enrichment was ~70% under all three drugs, glycolytic lactate could be enriched slightly less than 35% because of C1 decarboxylation in the hexose-monophosphate pathway. Thus, the lower enrichment values in the brain indicated the isotopic dilution of the glycolytic lactate by lactate from blood. Interestingly, brain lactate enrichment increased from pentobarbital to α-chloralose and morphine, thus demonstrating that more lactate was produced in the brain with cerebral activity (especially because blood lactate was twice as high under morphine). In fact, brain lactate production was more obvious in view of the lactate enrichments with glucose plus [3-13C]lactate infusion. Indeed, whereas blood lactate enrichment was 47.5, 50.2, or 30.3%, brain lactate enrichment was only 19.7, 11.2, or 10.3 under pentobarbital, α-chloralose, and morphine, respectively. This isotopic dilution indicated a higher contribution of the glycolytic lactate to the whole brain lactate pool. Using the specific enrichment of brain glucose C1 or C6 (equally labeled from liver gluconeogenesis) and that of brain and blood lactate, as shown in Equation 7,

\[
\alpha(\text{brain glucose C1 or C6}) + (100 - \alpha)\text{blood lactate C3} = 100\text{brain lactate C3} \quad (\text{Eq. 7})
\]

this percent contribution (\(\alpha\)) may be estimated. Then, the contribution was 62% under pentobarbital and 83% under α-chloralose. Under morphine, the contribution was 74% when blood lactate entry into the brain was about twice as high due to the higher blood concentration under that drug (30). Thus, assuming that blood lactate entry into brain would have been similar under the three drugs, the contribution of glycolytic lactate to the lactate pool would have been ~85% under morphine. These results emphasize therefore that the increase in brain lactate production and the increase in cerebral activity from pentobarbital to morphine (17, 22) were linked. The increased brain lactate concentration from pentobarbital to morphine was hence more likely due to this increase in lactate production than to changes in blood lactate influx.

The decrease in the enrichment ratio, \((\text{Ala C3})/\text{amino acid C_i})\), from pentobarbital to morphine observed with both labeled precursors (Table III) evidenced an increase in oxidative metabolism (via the tricarboxylic acid cycle). This result emphasized the relationship between metabolic and cerebral activities as discussed previously (22). However, the clear increase in amino acid labeling observed under the infusion of [1-13C]glucose plus lactate could not be related exclusively to the increase in metabolic activity from pentobarbital to morphine. Indeed, assuming unchanged contributions of blood glucose and blood lactate to the amino acid metabolism, the metabolism increase would also have stimulated amino acid labeling under the infusion of [1-13C]glucose plus lactate could not be related exclusively to the increase in metabolic activity from pentobarbital to morphine. Thus, importantly, these results revealed an increase in the contribution of brain lactate relative to the contribution of blood lactate to the brain metabolism from pentobarbital to morphine, concomitantly with the increase in metabolic activity. The consequences of the two effects on amino acid labeling were therefore either additive when using [1-13C]glucose or antagonistic when using [3-13C]lactate.

Under the infusion of [1-13C]glucose plus lactate, brain alanine C3 and brain lactate C3 were labeled to the same extent (Table II). This result is different from what generally occurs when [1-13C]glucose is given as the sole exogenous substrate. In the latter case, after 20 min of infusion alanine C3 is found to be less enriched than lactate C3 because of the lower enrichment rate of the alanine pool compared with that of lactate, which comes directly from glycolysis (19). Actually, the similar lactate and alanine enrichments evidenced the isotopic dilution of brain glycolytic lactate by lactate from blood. Under the infusion of glucose plus [3-13C]lactate, alanine C3 was more enriched than lactate C3. This result again demonstrated the occurrence of the brain endogenous lactate source and, importantly, that the labeled lactate from blood and the glycolytic lactate remained partly compartmentalized, leading, after metabolite extraction, to an apparent isotopic dilution of the labeled blood lactate as compared with its product, labeled alanine. These results indicated therefore that the glycolytic pyruvate formed inside the cells was not in equilibrium with the whole lactate pool through lactate dehydrogenase activity, in particular with the lactate from blood. This indicated that the latter was partly in the extracellular fluid.

Under the hyperglycemic and hyperlactemic conditions used in this study, there was no limitation to the supply of both glucose and lactate to the brain. In fact, the concentrations of these compounds in the brain were higher (~7–5 and 1–2 μmol/g wet weight from data in Table II, respectively, assum-
ing 100 mg of protein per gram) than under normal physiological conditions (2–3 and 1 μmol/g wet weight, respectively) (15). Moreover, the brain glucose/brain lactate concentration ratio was 5.8, 2.9, and 2.8 (from data in Table II) under pentobarbital, α-chloralose, and morphine, respectively; thus, in comparison to that of lactate, the oxidative metabolism of glucose

![FIG. 4. 13C-NMR spectra of brain extracts after a 20-min infusion of glucose with [3-13C]lactate. Spectra in panels A and B correspond to a few seconds and 2 min, respectively, after blood circulation arrest for rats under pentobarbital (p), α-chloralose (c), or morphine (m). Spectra are normalized relative to the inositol peaks (*). They show the time increases in alanine, lactate, glycerol-3-phosphate, and dihydroxyacetone phosphate signals. Peak assignments are as follows: peak 1, Ala C3; peak 2, lactate C3; peak 3, N-acetyl-aspartate C6; peak 4, GABA C3; peak 5, Gln C3; peak 6, Glu C3; peak 7, Gln C4; peak 8, Glu C4; peak 9, GABA C2; peak 10, taurine C2; peak 11, Asp C2; peak 12, creatine C2; peak 13, GABA C4; peak 14, N-acetyl-aspartate C3; peak 15, Asp C2; peak 16, N-acetyl-aspartate C2; peak 17, creatine C4; peak 18, Gln C2; peak 19, Glu C2; peak a, glycerol-3-phosphate C3; and peak b, dihydroxyacetone phosphate C3.

**TABLE III**

| Amino acid carbon AA Cᵢ | Pentobarbital | α-Chloralose | Morphine |
|-------------------------|--------------|--------------|----------|
|                         | [%] | [%] | [%] | [%] | [%] | [%] |
| [1-13C]glucose + lactate | 5.3 ± 0.7<sup>a</sup> | 2.19 ± 0.23 | 9.6 ± 0.7<sup>b</sup> | 1.98 ± 0.08<sup>b</sup> | 14.6 ± 1.1<sup>c</sup> | 1.56 ± 0.07<sup>c</sup> |
| Glu C4                  | 4.3 ± 0.7<sup>a</sup> | 2.88 ± 0.38 | 7.3 ± 0.6<sup>b</sup> | 2.71 ± 0.13<sup>b</sup> | 12.9 ± 1.2<sup>c</sup> | 1.78 ± 0.11<sup>c</sup> |
| GABA C2                 | 3.1 ± 0.4<sup>a</sup> | 4.60 ± 0.46 | 4.6 ± 0.5<sup>b</sup> | 4.80 ± 0.36<sup>b</sup> | 7.2 ± 0.9<sup>c</sup> | 3.44 ± 0.32<sup>c</sup> |
| Gln C4                  | 3.0 ± 0.4<sup>a</sup> | 4.84 ± 0.50<sup>a</sup> | 5.3 ± 0.3<sup>b</sup> | 4.00 ± 0.09<sup>b</sup> | 9.7 ± 0.9<sup>c</sup> | 2.44 ± 0.15<sup>c</sup> |
| Glucose + [3-13C]lactate| 10.0 ± 1.1 | 2.63 ± 0.19 | 9.3 ± 1.6 | 2.26 ± 0.28<sup>b</sup> | 14.6 ± 1.1<sup>c</sup> | 1.56 ± 0.07<sup>c</sup> |
| Glu C4                  | 7.0 ± 1.1 | 3.97 ± 0.48<sup>a</sup> | 7.6 ± 1.4 | 2.85 ± 0.39<sup>b</sup> | 6.5 ± 1.1 | 2.02 ± 0.24<sup>c</sup> |
| GABA C2                 | 4.8 ± 0.6 | 6.32 ± 0.56<sup>a</sup> | 4.6 ± 0.8 | 5.29 ± 0.33<sup>b</sup> | 4.3 ± 0.6 | 3.41 ± 0.30<sup>c</sup> |
| Gln C4                  | 4.7 ± 0.7 | 6.50 ± 0.73<sup>a</sup> | 5.3 ± 1.4 | 4.40 ± 0.96<sup>b</sup> | 5.4 ± 0.9 | 2.53 ± 0.30<sup>c</sup> |

<sup>a</sup> Significant difference between pentobarbital and α-chloralose.
<sup>b</sup> Significant difference between α-chloralose and morphine.
<sup>c</sup> Significant difference between morphine and pentobarbital.
should have been more favored under pentobarbital than under α-chloralose or morphine. Paradoxically, the brain amino acid labeling pattern reflected exactly the contrary, i.e. the relative contribution of blood glucose to amino acid labeling through the tricarboxylic acid cycle activity (Asp, Gln, Glu, and GABA, whose labeling is mainly due to neuronal metabolism) increased from pentobarbital to α-chloralose and morphine. This was not consistent with a simple competition between the neuronal glycolytic pyruvate and the pyruvate resulting only from blood lactate oxidation by neuronal lactate dehydrogenase to account for the acetyl-CoA sources in the neuronal compartment. There might be a competition between the neuronal glycolytic pyruvate and another pyruvate source. The sole potential pyruvate source was the lactate endogenous to brain, providing that the latter was synthesized in a compartment other than the neurons. As evidenced previously from acetyl-CoA enrichment analysis (17), the astrocytes were mainly responsible for the brain lactate production, a finding in agreement with the much more efficient lactate production by astrocytes than by neurons (15). Therefore, the amino acid labeling patterns could be due to a progressively more pronounced involvement of astrocytic glycolytic lactate than of blood lactate in the move from pentobarbital to morphine. This mechanism would also explain the higher alanine C3 than lactate C3 enrichment under the infusion of glucose plus [3-13C]lactate. Indeed, assuming that alanine was mostly labeled in the astrocytic compartment when the astrocytes are known to produce lactate (15), a higher alanine than lactate enrichment would be unlikely. On the contrary, if alanine was labeled in neurons from blood-labeled lactate, the higher alanine than lactate enrichment could be due to the isotopic dilution of lactate from blood by glycolytically lactate produced in the astrocytes.

These conclusions emphasize the observation that despite the high brain lactate content linked to hyperlactemia, the astrocytic redox and pH conditions remained favorable to glycolytic lactate production. Moreover, the fact that the brain glycolytic lactate and lactate from blood did not mix in a single pool indicated that this latter was mostly excluded from the astrocytic compartment. This suggested that, although blood lactate entry into brain parenchyma likely occurred via the astrocytic end feet, the transmembrane gradient and pH conditions favored its rapid exclusion from the astrocytes and its subsequent uptake by neurons through the different monocarboxylate transporters MCT1 and MCT2, respectively (31, 32). As the result of the lactate consumption in neurons, the lactate gradient between neurons and astrocytes would be maintained, which would constitute the driving force for the move of lactate from astrocytes to neurons, thus making possible the regeneration of NAD+ to fulfill the astrocytic energy demand from glycolysis. On the other hand, the increased neuronal use of blood and astrocytic glycolytic lactate as neuronal oxidative substrates with brain activity emphasizes the possibility of the redox switch in neurons as proposed (33). Indeed, these authors evidenced that the two pyruvate species, the glycolytic pyruvate and the pyruvate synthesized from exogenous lactate by lactate dehydrogenase, were compartmentalized and that the production of the first was inhibited when the second was used preferentially as an oxidative substrate. Moreover, the fact that alanine was synthesized in neurons suggested that it could be involved in the alanine-lactate shuttle between neurons and astrocytes (34, 35), which might contribute to regenerating the cytoplasmic NAD+ in astrocytes through NADH oxidation by the respiratory chain in neurons.

Conclusion—The role of lactate in brain metabolism is a topical issue that has recently been reviewed (36). In that article, the authors critically review the experimental evidence and theoretical arguments sustaining either the conventional view that glucose is the metabolic substrate of the mature brain or that lactate is the substrate of active neurons according to the ANLSH. They emphasize three main points as follows: 1) the site and mode of glucose use; 2) the site of lactate production and use; and 3) the timing of lactate use. As regards the last point, an intrinsic limitation in our work is that the 13C enrichments measured in metabolites reflect the accumulation of the isotope over a large time scale, thus precluding any information on the timing of glucose and lactate metabolism. Another limitation is that the study was performed on whole brain, i.e. at a macroscopic scale, when tight astrocyte-neuron interactions at the microscopic level could presumably be involved in the ANLSH. This probably constitutes a limitation for most, if not all, metabolic approaches, which cannot demonstrate whether the neuronal lactate metabolism results from the diffusion of lactate in the extracellular fluid or from a specific interaction between the activated neurons and the astrocytes in their close environment. However, under our experimental conditions, we provide evidence that both the brain metabolic activity and the contribution of blood glucose relative to the contribution of blood lactate to the brain metabolism were linked to cerebral activity. This evidence indicates that when glucose and another substrate for the oxidative metabolism (in the present case lactate) are present in blood as two competing substrates to fuel brain metabolism, glucose is the one whose consumption is related to cerebral activity, thus eliciting its crucial role as the primary brain metabolic fuel. More importantly, we demonstrate that the brain glycolytic activity leading to lactate production mainly occurs outside the neuronal compartment, very likely in the astrocytes, and that neurons use the lactate generated from these cells as metabolic fuel. Although our study was performed with rats under the effect of depressant drugs, the conclusions drawn from the results appear to be in agreement with the elementary steps of lactate production by astrocytes and lactate consumption by neurons as described in the ANLSH.

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