Oxygen and Glucose Consumption Related to Na⁺–K⁺ Transport in Canine Brain

JENS ASTRUP, M.D., PER MØLLER SØRENSEN, M.D., AND HANS RAHBEK SØRENSEN, M.D.

SUMMARY This study examines the relation between Na⁺–K⁺ transport and metabolism in the canine brain. Cerebral oxygen and glucose consumption was measured by the sagittal sinus outflow technique. Synaptic transmission and related metabolism was blocked by pentobarbital 40 mg/kg (EEG flat). Lidocaine blocked an additional 15–20%, presumably by restricting Na⁺–K⁺ leak fluxes and reducing the demand for Na⁺–K⁺ transport. Ouabain blocked an additional 20–25% of metabolism. Ouabain also inhibited the Na⁺–K⁺ sensitive ATPase associated transport and caused a net efflux of K⁺ from the cellular compartment as evidenced by an increasing extracellular K⁺ concentration in the cortex. Accordingly, a total of 40% of metabolism in the EEG-arrested barbiturate inhibited brain could be related to Na⁺–K⁺ leak fluxes and associated transport. The remaining 60% are related to processes unidentified by this study. It is concluded that cerebral metabolism may be reduced below the hitherto described barbiturate minimum.

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THIS STUDY seeks a quantitative evaluation of the energy consumed by Na⁺–K⁺ transport in the brain. Particular reference is attached to the Na⁺–K⁺ leak fluxes and associated active transport which occur in the EEG-arrested brain. Synaptic transmission was blocked by pentobarbital to obtain a flat EEG. The effects of lidocaine and of ouabain on cerebral oxygen and glucose consumption were then measured using the sagittal sinus outflow technique in dogs on cardiopulmonary bypass circulation. In line with previous studies, it was assumed that lidocaine restricts Na⁺–K⁺ leak fluxes by blocking membrane permeability, and, by this effect, reducing the demand for Na⁺–K⁺ transport. The remaining Na⁺–K⁺ transport was blocked by ouabain, a specific Na⁺–K⁺ sensitive ATPase inhibitor. Ouabain was injected into the systemic circulation but crossed the blood-brain barrier and caused ion transport inhibition. This effect was shown by measurements of the extracellular K⁺ concentration in the cerebral cortex by potassium electrodes.

Methods

Twelve mongrel dogs weighing 23–38 kg were anesthetized by thiopental 17–27 mg/kg (mean 20.8 mg/kg) intravenously. Endotracheal intubation was performed after muscular relaxation using gallamine 2–8 mg/kg. Respiration was controlled by mechanical ventilation. Anesthesia was maintained with halothane 1–1.5% mixed in 25/75% oxygen/air. During cardiopulmonary bypass circulation this gas mixture was led through the bubble-oxygenator (Venoterm 5000) primed with Haemaccel (Haemaccel is a colloidal plasma substitute, 1 ml containing 35 mg polygeline, 8.5 mg NaCl, 0.38 mg KCl, 0.7 mg CaCl₂) 1000 ml and Ringer’s solution 300 ml to obtain hemodilution. The oxygenated blood was pumped by roller-pump into the animal via the cannula in the left femoral artery. Heparinization was induced by heparin 3 mg/kg and maintained by 1 mg/kg/h. Cardioplegia was obtained by flushing the coronary circulation using potassium chloride 1 M after clamping the ascending aorta.

Cardiopulmonary bypass circulation was maintained at a flow rate of 100 ml/kg/min. BP was controlled within 50–100 mm Hg by occasional injection of methoxamine 0.5–1 mg or chlorpromazine 1–2 mg. Brain temperature was maintained at 37°C by adjusting the water temperature in the heat exchanger of the oxygenator.

Cerebral blood flow was measured according to the method of Rapela et al. using some of the modifications described by Michenfelder et al. The bone flap over the superior sagittal sinus was isolated except for the very thin parts forming the floor in the frontal sinuses. Bleeding from the diploic veins in the skull was controlled by bone wax. The ethmoidal veins were ligated to prevent escape of venous outflow from the brain by that route. The occipital part of the superior sagittal sinus was cannulated using a metal cannula of 2 mm ID. The sinus was occluded distal to the cannula by compression and ligation. Two dural incisions were placed parallel to the sinus to allow its collapse. After
these precautions the sinus outflow was little affected by changes in outflow resistance (catheter level 10 cm above or below sinus level). These precautions were considered necessary to ensure venous outflow from a constant part of the brain (see discussion by Michenfelder et al.). Cerebral blood flow (CBF) was then measured as venous outflow. The cerebral metabolic rate (CMR) of oxygen (CMRO$_2$) and glucose (CMRgluc) in the drained portion of the brain were calculated from blood flow and arteriovenous differences of oxygen (AVDO$_2$) and glucose (AVDgluc).

This method yields changes in cerebral blood flow and metabolism in each animal during the course of the experiment. Absolute values as related to unit brain weight cannot be calculated unless the weight of the drained portion of the brain is estimated. According to Michenfelder et al., about 40-45% of the brain is drained by this method.

Oxygen content of the blood was calculated from measurements of hemoglobin concentration and oxygen saturation (Radiometer OSM$_4$O equipment) adding the dissolved amount of oxygen calculated from the oxygen tensions by using a solubility constant of 0.022 (37°C) ml O$_2$/760 mm Hg/ml blood. Whole blood glucose concentration (BG) was measured in triplicate using a standard technique.

A needle thermistor was inserted into the left hemisphere to ensure correct brain temperature. The craniectomy over the right parietal region was enlarged. The dura was opened, and a surface electrode placed so that it just touched the brain surface. Two chlorinated silver wires were placed on the dura to obtain the EEG signal.

The surface potassium electrode consisted of a polyvinyl-chloride membrane soaked with valinomycin (Radiometer Selectrode). It was modified with a "built-in" reference (saline-silver-chloride junction). The electrode was calibrated in KCl 2, 3, 5, 100 mM plus NaCl 148, 147, 145, 50 mM respectively, using KCl 100 mM plus NaCl 50 mM as an internal solution. The satisfactory correlation between the surface electrode and a microelectrode inserted in the cerebral cortex has been described previously.2, 4

Groups of 6 animals permitted use of the "sign-test," by which a change in the same direction in all 6 animals is significant at the p < 0.05 level.

Experimental Procedures

Measurements of blood flow, blood gases, hemoglobin, AVDO$_2$, and AVDgluc were performed at 5 min intervals. Two groups of animals were studied using the following procedures:

1. Pentobarbital 40 mg/kg.* Pentobarbital was given by constant rate infusion over a 15-25 min period. During this procedure the EEG became flat (defined as absence of bursts for more than one min). Six dogs were studied. Brain temperature was 37°C.

2. Lidocaine 160 mg/kg subsequent to pentobarbital.* Ten min after the pentobarbital infusion, lidocaine was infused over a 20-35 min period during which the EEG remained flat.

3. Ouabain 100 mg/kg subsequent to lidocaine. In another 6 dogs the effect of ouabain was studied subsequent to lidocaine. Lidocaine 160 mg/kg was given as a bolus in the pump reservoir. When the drug reached the animal, the EEG became flat within seconds. No spike activity occurred. After a steady state period, ouabain 100 mg/kg dissolved in saline was given as a bolus in the pump reservoir. The surface potassium concentration (K$^+)$ was monitored and the EEG remained flat.

Results

Physiological Variables

The measured physiological variables prior to and after injection of drugs are summarized in table 1.

*This group of 6 dogs illustrates the interaction between pentobarbital and lidocaine, and the data are contained in reference 4 (Astrup et al.) in which a full account of the mode of action of these 2 drugs is given. The data also illustrate some of the energy requiring processes in the brain and their possible inhibition, and as such they are included in the present study.
TABLE 2  Effects of Pentobarbital, Lidocaine, and Ouabain on Cerebral Oxygen and Glucose Consumption X ± SEM 37°C

<table>
<thead>
<tr>
<th>Substance</th>
<th>CMRO₂ %</th>
<th>CMRGluc %</th>
</tr>
</thead>
<tbody>
<tr>
<td>halothane</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>pentobarbital</td>
<td>-30.5*</td>
<td>-25.8*</td>
</tr>
<tr>
<td>n = 6</td>
<td>5.5</td>
<td>6.1</td>
</tr>
<tr>
<td>lidocaine</td>
<td>-15.2*</td>
<td>-19.0*</td>
</tr>
<tr>
<td>n = 6</td>
<td>3.4</td>
<td>5.3</td>
</tr>
<tr>
<td>ouabain</td>
<td>-23.7*</td>
<td>-35.8*</td>
</tr>
<tr>
<td>n = 6</td>
<td>4.7</td>
<td>2.4</td>
</tr>
</tbody>
</table>

*p < 0.05, sign-test.
*The pentobarbital and lidocaine data have been previously published.

Pentobarbital 40 mg/kg

Pentobarbital reduced the CMR and this effect reached a maximum when the EEG became flat.7,4 The maximum reduction in the CMR was 25–30% when halothane anesthesia was used alone (table 2). The CBF fell according to BP (unchanged CVR). The fall in CBF was relatively larger than the reduction of the CMR as indicated by the increase in AVDO₂ (table 1).

Lidocaine 160 mg/kg

In the brain in which metabolism was suppressed and synaptic transmission abolished by pentobarbital, lidocaine caused an additional reduction of the CMR of 15–20% (table 2). Lidocaine increased CVR. The fall in CBF was relatively larger than the reduction of the CMR (increasing AVDO₂) (table 1).

Ouabain 100 mg/kg

The rise in (K⁺)₅ (fig. 1) affirms ion pump inhibition by ouabain and net efflux of potassium. Following injection of ouabain the CMR was reduced by an additional 25–35% over that caused by lidocaine inhibition (table 2). The fall in CMRO₂ is also evidenced by the increase in sagittal venous Po₂ and fall in AVDO₂, which is only partly accounted for by the increase in CBF (table 1).

Only the first set of measurements (within 5 min after ouabain injection) was included for analysis as ouabain caused adverse systemic effects. Severe hypertension and metabolic acidosis developed within 15–20 min. The CMR recovered toward the control level. At this point (K⁺)₅ had increased to 10–20 mM (fig. 1). This secondary increase in CMR during ouabain inhibition may be accounted for by activation of uninhibited ATPase through excess extracellular K⁺.

Discussion

Results of this study indicate that the total energy consumption in the brain is a sum of at least 3 portions (fig. 2). One portion relates to synaptic transmission. It is coupled to function, and it is assumed to be completely inhibited in all conditions of a flat EEG irrespective of the cause. The second portion is related to residual Na⁺-K⁺ leak fluxes and associated ion transport in the EEG arrested brain. As shown in the present study, this portion can be inhibited by

![Figure 1](http://stroke.ahajournals.org/)

**Figure 1.** The increasing (K⁺)₅ indicates net K⁺ efflux due to ouabain inhibition of Na⁺-K⁺ transport. The K⁺ efflux rates were slightly slower than the efflux rates during ischemia (hatched area). Time zero indicates ouabain injection, and in the previous experiments onset of circulatory arrest.

![Figure 2](http://stroke.ahajournals.org/)

**Figure 2.** Energy consuming processes in the brain: 1) Energy consumption by synaptic transmission and associated Na⁺-K⁺ fluxes are related to the functional state. 2) In the functionally arrested brain (flat EEG) Na⁺-K⁺ leak fluxes and associated active transport consumes about 40% of the residual energy production. 3) The remaining 60% are consumed by unidentified processes.
lidocaine and ouabain. The third portion is related to hitherto unidentified processes. Barbiturates inhibit portion one, lidocaine inhibits portion one and part of portion 2, and ouabain inhibits the remainder of portion 2, and hypothermia provides a partial and non-specific inhibition of all 3 portions. This general outline of cerebral energy consumption may provide a rational approach to the problem of protection of the ischemic brain by metabolic inhibition.

Energy Consumption Related to Synaptic Transmission

This portion of energy consumption is coupled to function. It is subjected to large variations, depending on the functional state of the brain, and it is completely inhibited in conditions causing a flat EEG. This portion of the energy consumption, which is believed to be related to the intensity of synaptic transmission and associated Na⁺-K⁺ fluxes,

* has been studied in relation to brain function. The mode of action of barbiturates, and the effect of barbiturates in combination with other conditions of a flat EEG, in particular ischemia, need reconsideration. Barbiturates inhibit cerebral metabolism, and as shown by Michenfelder, the effect of thiopental is at a maximum when the EEG becomes flat. Pentobarbital has a similar function-metabolism-coupled mode of action. This mode of action suggests that the metabolic effect of barbiturates relates specifically to the inhibition of synaptic transmission. This implies 2 things: First, that barbiturates have no additional metabolic effect when given in conditions of a preexisting flat EEG. Second, that such conditions causing a flat EEG inhibit cerebral metabolism by a similar amount, i.e., have a “barbiturate-like” effect. These implications also hold with lidocaine. As previously found, pentobarbital has no additional metabolic effect when given to the lidocaine arrested brain with a flat EEG. Furthermore, lidocaine was found to be as effective as pentobarbital as a metabolic depressant. However, lidocaine has a membrane effect which causes metabolic inhibition in addition to the inhibition related to suppression of synaptic transmission. Barbiturates are without such a membrane effect. This was evidenced by previous studies, which failed to show any effect of phenobarbital on the initial rate of efflux of potassium in the ischemic brain, indicating barbiturate-unaffected Na⁺-K⁺ membrane permeabilities. These results support the concept that the metabolic effect of barbiturates is probably related specifically to inhibition of synaptic transmission. This point justifies the use of barbiturates as a means to block synaptic transmission for studies of the EEG-arrested brain.

Other conditions causing a flat EEG may have a barbiturate-like effect on metabolism. As mentioned, lidocaine has a barbiturate-like effect on brain metabolism, which relates to arrest of EEG activity. Also, cerebral ischemia is associated with a flat EEG. It is of interest to the problem of protection for the ischemic brain through metabolic inhibition, to know whether or not the demand for energy in the ischemic tissue is reduced by abolishing synaptic transmission, i.e., a “barbiturate-like” effect of the ischemia itself. This problem remains unanswered, but there is evidence suggesting that energy demand is reduced at the point of electrical failure in the ischemic brain. Studies of incomplete ischemia have revealed 2 flow thresholds, the upper of about 18–16 ml/100 g • min being related to failure of synaptic transmission, and the lower of about 10–8 ml/100 g • min being related to failure of the ion pump and energy metabolism. At flow levels between these 2 thresholds, synaptic transmission is abolished while energy metabolism is grossly preserved. This indicates a maintained balance between oxygen availability, which is low, and metabolic demand. Consequently, the metabolic demand must be low as well, indicating a “barbiturate-like” effect of ischemia itself. Thus protection of the ischemic brain by way of metabolic inhibition with the use of barbiturates seems irrational. This argument has previously been discussed by Michenfelder and Theye and Cohen.

Energy Consumption Related to Na⁺-K⁺ Leak Fluxes and Associated Ion Transport

The results in table 2 suggest that the leak fluxes of Na⁺-K⁺ and associated transport consume about 40% of the total energy production in the EEG-arrested brain (fig. 2). This part was identified as the lidocaine plus ouabain inhibitable part of the residual oxygen and glucose consumption in the EEG-arrested brain. This result corresponds well to results obtained in non-stimulated electrically silent brain slices. Whittam found that ouabain inhibited oxygen consumption rate by 50%. Lidocaine and ouabain have different modes of action. As a local anesthetic lidocaine blocks the sodium channels in nerve membranes. Previous studies suggest that lidocaine has a similar action in the brain as evidenced by restriction of sodium and potassium ion fluxes. In the ischemic brain this effect appears as a delayed potassium efflux and slowing of the increase in the extracellular potassium concentration in the cortex. In the non-ischemic brain this effect appears as abolition of synaptic transmission as well as additional metabolic inhibition in the barbiturate inhibited EEG-arrested brain. The latter effect is explained by reduction of the load on the ion transport by restriction of Na⁺-K⁺ leak fluxes. The potassium efflux in the ischemic brain is only delayed and not completely prevented. This indicates that the Na⁺ channels are only incompletely blocked by lidocaine. It was assumed that a portion of the Na⁺-K⁺ leak fluxes, with associated energy consuming Na⁺-K⁺ transport, remains in the lidocaine-inhibited brain. Accordingly, it was postulated that a block of this Na⁺-K⁺ transport by ouabain would reduce oxygen and glucose consumption and release net Na⁺-K⁺ leak fluxes causing a net accumulation of potassium in the extracellular space. That ouabain in the injected dose crossed the blood-brain barrier in sufficient amounts to inhibit the Na⁺-K⁺-ATPase is demonstrated by the immediate fall in CMRO₂ and
CMRgluc (table 2) and by the net potassium efflux (fig. 1). The potassium efflux was slower during ouabain than during ischemia. If the membrane permeabilities for Na\(^+\)-K\(^+\) and the net water movements are comparable, then the Na\(^+\)-K\(^+\) transport is more effectively inhibited by ischemia than by ouabain. The overall effects of ouabain are complex including severe metabolic acidosis, increased vascular resistance and hypertension. In the brain the metabolic inhibition was only transient and it recovered in some animals within 10-15 min. The increased amounts of K\(^+\) in the extracellular space may compete with ouabain for binding sites on the Na\(^+\)-K\(^+\) ATPase. This explains the metabolic recovery as reactivation of part of the Na\(^+\)-K\(^+\) transport and justifies selection of the initial metabolic inhibition as the true indicator of ouabain Na\(^+\)-K\(^+\) transport inhibition.

Lidocaine and ouabain inhibited about 40% of metabolism in the arrested brain. Consequently, the Na\(^+\)-K\(^+\) leak fluxes and associated transport probably consume 40% of the energy production by oxidative metabolism in the pentobarbital inhibited EEG-arrested brain.

**Energy Consumption Associated with Hitherto Unidentified Processes**

Metabolic processes of yet unidentified nature consume about 60% of the residual energy production in the EEG-arrested brain. Hypothermia inhibits this portion but no specific methods are yet available for its inhibition.

**Protection by Metabolic Inhibition**

Use of barbiturates for cerebral protection to produce metabolic inhibition seems irrational, since ischemia itself has a "barbiturate-like" effect on metabolic demand. Both conditions abolish synaptic transmission and reduce metabolic demand. Other effects than metabolic inhibition must be considered as an explanation of the possible barbiturate protection in focal cerebral ischemia, e.g., the hemodynamic effect of inverse-steal. Lidocaine is an interesting candidate for protection in complete global cerebral ischemia. The lidocaine effect resembles the effect of hypothermia in 2 ways: Both conditions cause a delay in the ischemic K\(^+\) efflux, as well as additional metabolic inhibition in the EEG-arrested brain, and the effects are additive. It is yet undetermined if the protective action of hypothermia in cerebral ischemia is related to these effects. It is possible that lidocaine may provide hypothermia-like protection, or may improve protection by hypothermia. If the protective action of hypothermia relates to inhibition of the hitherto unidentified but lidocaine-insensitive processes, lidocaine is an unlikely candidate for protection.

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J Astrup, P M Sørensen and H R Sørensen

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