Activation-Induced Resetting of Cerebral Metabolism and Flow Is Abolished by β-Adrenergic Blockade With Propranolol

Ina K. Schmalbruch, MD, PhD; Rasmus Linde, PhD; Olaf B. Paulson, MD, DMSc; Peter L. Madsen, MD, DMSc

Background and Purpose—It has previously been shown that activation will increase cerebral blood flow (CBF) and cerebral glucose uptake (CMRglc) in excess of cerebral oxygen uptake (CMRO2). Our purpose was to investigate the influence of β-adrenergic blockade with propranolol on the activation-induced uncoupling of cerebral glucose and oxygen metabolism.

Methods—Using awake rats, we determined the cerebral arteriovenous differences of oxygen [(a−v)O2], glucose [(a−v)glc], and lactate [(a−v)lac] both under baseline conditions and during activation. The molar ratio between CMRO2 and CMRglc, the oxygen-glucose index (OGI), was calculated.

Results—Without β-adrenergic blockade, activation decreased the (a−v)O2 but not the (a−v)glc, reducing the OGI from 6.1 during baseline conditions to 4.0 under activation (P<0.01). The (a−v)O2 decreased, indicating that the ratio CBF/CMRO2 had increased. Under baseline conditions, a slight flux of lactate from the brain was observed. Activation increased the arterial lactate concentration, and during this condition, the lactate flux from the brain was reversed into a slight lactate uptake. Propranolol administration did not change the behavior of the animals during activation. After administration of propranolol, baseline values were unaffected, but β-adrenergic blockade totally abolished the activation-induced uncoupling of (a−v)O2 from (a−v)glc, because both remained constant with an unchanged OGI. The unchanged (a−v)O2 indicates that CBF remained unchanged compared with CMRO2.

Conclusions—β-Adrenergic blockade by propranolol abolishes the activation-induced uncoupling of cerebral oxygen to glucose metabolism and the changes in (a−v)O2. This may be of most significance to studies of cerebral activation by the blood oxygen level–dependent fMRI method. (Stroke. 2002;33:251-255.)

Key Words: cerebral blood flow ■ cerebral cortex ■ cerebral metabolism ■ glucose ■ lactates ■ oxygen ■ propranolol

Cerebral activation will increase cerebral blood flow (CBF) and cerebral metabolic rate of glucose (CMRglc) more than it will increase the cerebral metabolic rate of oxygen (CMRO2). This so-called uncoupling phenomenon has been observed in the human setting1–6 as well as in the animal setting.7,8 Another line of investigation showed that severe immobilization stress in rats will increase global CMRO2 and flow by 80%,9 and it has been demonstrated that this cerebral activation response can be totally abolished by β-adrenergic blockade with propranolol.9 It remains unknown, however, whether β-adrenergic blockade hinders the changes in the metabolic patterns during activation or whether it only blocks the general increase in flow and oxygen consumption observed during immobilization stress. This question was the basis for the present investigation.

Two theories may thus be advanced regarding these effects of β-adrenergic blockade: (1) β-adrenergic blockade has an isolated influence on the cerebral vasomotor function, abolishing flow increase during activation, but has no influence on cerebral metabolism and is without influence on the activation per se, ie, on the behavior during activation; (2) β-adrenergic blockade influences both vasomotor function and cerebral metabolism without affecting behavior during arousal.

To investigate these 2 hypotheses, the present study was undertaken in awake rats and used arteriovenous (a−v) differences to determine possible changes in the metabolic pattern during activation. In addition, the animals’ behavior was observed to note any changes in arousal pattern.

Materials and Methods

Animals and Surgical Procedures
Male Sprague-Dawley rats (Møllegaarden, Lille Skensved, Denmark) 3 months old (body weight 400 to 450 g) were used. The animals were maintained on an alternate 12-hour light/dark cycle, with humidity controlled at normal levels. Access to water and food was unlimited. All procedures performed on animals were in strict accordance with the National Institutes of Health Guide for Care and
Figure 1. OGI determined before, during, and after activation. No propranolol was administered. The first 3 paired measurements were obtained with the experimental animal in the shelter box. After opening of the box (first vertical bar), 3 paired blood samples were obtained. After 10 minutes’ activation, the box was closed (second vertical bar), and 3 paired blood samples were obtained with the animal in the shelter box. Values represent the mean of measurements in a group of 6 animals. Error bars represent SD.

Figure 2. OGI determined before, during, and after activation. Measurements were performed after administration of propranolol. The first 3 paired measurements were obtained with the experimental animal in the shelter box. After opening of the box (first vertical bar), 3 paired blood samples were obtained. After 10 minutes’ activation, the box was closed (second vertical bar), and 3 paired blood samples were obtained with the animal in the shelter box. Values represent the mean of measurements in a group of 7 animals. Error bars represent SD.

Use of Laboratory Animals and were approved by the Animal Experimentation Inspectorate. To accustom the animal to the experimental setting, they spent a minimum of 1 day in their cages in the laboratory setting. To stabilize arterial glucose levels during activation, rats were fasted for 20 hours before the experiment. Access to water was unrestricted. All surgical procedures to be described were performed at the same time of day.

On the day of the experiment, the animal was anesthetized with halothane (4% for induction and 1.5% for maintenance) in 70% N2O/30% O2. Polyethylene catheters (length 18 cm, PE 50, Portex) filled with physiological saline with 30 U sodium heparin/mL, were inserted into both femoral arteries and femoral veins. Both femoral nerves were cut to partially immobilize the hind limbs. A local anesthetic (lidocaine + norepinephrine 10 mg/mL) was instilled into the wounds, which were sutured and covered with an anesthetic lidocaine-prilocaine lotion (EMLA creme, Astra).

Cerebral venous blood was obtained through a catheter that was inserted into the confluence of sinuses through a screw placed just posterior to the lambdoid suture. The cerebral venous catheter was identical to the catheters inserted into the femoral vessels. Special care has to be taken when sampling blood from the confluence of sinuses to avoid contamination of extracerebral venous blood, ie, retrograde flow from the larger neck vessels into the confluence of sinuses, as previously extensively investigated by our group. Thus, the rate of sampling was in all cases <0.57 mL/min.

A plaster cast was applied around the lower torso for restraint, and the rat was left undisturbed for recovery. In the recovery period, the rat was kept in a shelter specially designed to minimize external stimulation. The shelter was a closed triangular box with apertures permitting passage of blood sample catheters. In the shelter, the rat was monitored by closed-circuit video surveillance, and a small ventilator ensured air change. Rectal temperature was monitored with a rectal probe, and mean arterial blood pressure was continuously measured with a pressure transducer connected to one of the arterial catheters.

Experimental Protocols
The animal remained undisturbed for 200±20 minutes (mean±SD) before initiation of the experiment. Measurements were performed during 3 different conditions.

Baseline Conditions
Before activation, 3 paired blood samples were obtained with the animal remaining in the closed shelter box (Figures 1 and 2). During baseline conditions, external stimulation was kept to a minimum.

Activation
Opening of the shelter box and exposing the rat to the environment induced 10 minutes of cerebral activation. No further activation procedures were performed. Three paired blood samples were obtained during the activation period (Figures 1 and 2).

Postactivation
During the 35 minutes after the activation period, 3 paired blood samples were obtained exactly as before activation (Figures 1 and 2).

The arterial oxygen tension (PaO2), pH, and arterial carbon dioxide tension (PaCO2) were measured in blood samples taken from the right femoral artery in the middle of each of the 3 periods of measurement.

Two groups of animals were investigated: Group 1 (6 young adult rats) was activated as described above. Group 2 (7 young adult rats) received propranolol (propranolol hydrochloride, 2 to 3 mg/kg IV. Inderal, 1 mg/mL in saline) before activation. To avoid changes in mean arterial blood pressure of >5 mm Hg, propranolol was slowly injected over a period of 60 minutes (range 48 to 74 minutes). After administration of propranolol, the rats in group 2 were activated exactly as described for group 1.

Blood Sampling
Blood for determination of cerebral arteriovenous differences for oxygen, glucose, and lactate [(a–v)O2, (a–v)glc, and (a–v)lac] was obtained from paired blood samples. The volumes of the arterial and cerebral venous catheter systems were 0.055 mL, and before blood sampling, a dead-space volume of 0.150 mL blood was drawn. Immediately after this, 0.30 mL of blood was drawn into ice-cold 0.5-mL glass microtiter syringes (Hamilton Bonaduz AG) prepared with heparin and NaF. The rate of sampling was the same from the arterial and the cerebral venous catheters and in all cases was <0.57 mL/min. After completion of each paired blood sample, blood loss was replaced by systemic intravenous injection of blood obtained from a donor rat of the same breed. Only blood from fasting rats killed <30 minutes before transfusion was used.

Immediately after blood sampling, 0.18 mL blood for determination of whole-blood glucose and lactate concentrations were transferred from the syringes to vials containing dried fluoride-EDTA. These vials were stored on ice and analyzed in quadruplicate within 60 minutes on a YSI 2700 Select Biochemistry Analyzer (Yellow Springs Instruments Co Inc). Approximately 0.12 mL blood for determination of (a–v)O2 was stored on ice under strict anaerobic conditions and analyzed in triplicate within 40 minutes on an OSM3 apparatus (Radiometer).

Monitoring Motor Activity
To evaluate the motor activity of our animals, videotapes obtained from 15 minutes before the experiment were analyzed and different types of behavior were documented. We evaluated the number of body movements and the number of sniffings. Values were calcu-
Table 1. Cerebral Arteriovenous Differences and Physiological Variables Obtained Without Administration of Propranolol

<table>
<thead>
<tr>
<th>No Propranolol</th>
<th>Baseline (n=6)</th>
<th>Activ. (n=6)</th>
<th>Postactiv. (n=6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A\textsubscript{O2}, mmol/L</td>
<td>8.49 ± 0.45</td>
<td>8.67 ± 0.27</td>
<td>8.28 ± 0.31</td>
</tr>
<tr>
<td>A\textsubscript{glc}, mmol/L</td>
<td>5.68 ± 0.72</td>
<td>6.58 ± 0.63*</td>
<td>6.81 ± 0.83†</td>
</tr>
<tr>
<td>A\textsubscript{lac}, mmol/L</td>
<td>0.57 ± 0.41</td>
<td>1.85 ± 0.60†</td>
<td>0.74 ± 0.24</td>
</tr>
<tr>
<td>Hemoglobin, mmol/L</td>
<td>9.04 ± 0.45</td>
<td>9.11 ± 0.26</td>
<td>8.78 ± 0.35</td>
</tr>
<tr>
<td>Mean arterial blood pressure, mm Hg</td>
<td>121 ± 5.0</td>
<td>129 ± 7.0</td>
<td>124 ± 7.0</td>
</tr>
<tr>
<td>PacO\textsubscript{2}, mm Hg</td>
<td>40.6 ± 2.1</td>
<td>36.9 ± 2.7*</td>
<td>37.6 ± 2.6</td>
</tr>
<tr>
<td>(a−v)\textsubscript{O2}, mmol/L</td>
<td>3.65 ± 0.45</td>
<td>2.72 ± 0.41†</td>
<td>3.53 ± 0.28</td>
</tr>
<tr>
<td>(a−v)\textsubscript{glc}, mmol/L</td>
<td>0.63 ± 0.08</td>
<td>0.71 ± 0.13</td>
<td>0.63 ± 0.03</td>
</tr>
<tr>
<td>(a−v)\textsubscript{lac}, mmol/L</td>
<td>-0.05 ± 0.04</td>
<td>0.02 ± 0.05†</td>
<td>-0.04 ± 0.02</td>
</tr>
<tr>
<td>OGI</td>
<td>6.1 ± 0.9</td>
<td>4.0 ± 0.9†</td>
<td>5.8 ± 0.5</td>
</tr>
<tr>
<td>Movements, min</td>
<td>0.1 ± 0.2</td>
<td>0.4 ± 0.6*</td>
<td>0.0 ± 0.1</td>
</tr>
<tr>
<td>Sniffing, min</td>
<td>1.6 ± 0.7</td>
<td>5.3 ± 2.7†</td>
<td>1.6 ± 0.9</td>
</tr>
</tbody>
</table>

Values are mean ± SD. The values represent the mean of values obtained in 6 animals. These animals were studied during identical conditions, during baseline conditions (Baseline), during activation (Activ.), and in a period after activation was terminated (Postactiv.).

Statistical significance is denoted by *P<0.05; †P<0.01.

Table 2. Cerebral Arteriovenous Differences and Physiological Variables Obtained After Administration of Propranolol

<table>
<thead>
<tr>
<th>With Propranolol</th>
<th>Baseline (n=7)</th>
<th>Activ. (n=7)</th>
<th>Postactiv. (n=7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A\textsubscript{O2}, mmol/L</td>
<td>7.60 ± 1.84</td>
<td>7.64 ± 1.99</td>
<td>7.53 ± 1.83</td>
</tr>
<tr>
<td>A\textsubscript{glc}, mmol/L</td>
<td>5.80 ± 0.45</td>
<td>6.12 ± 0.62*</td>
<td>6.16 ± 0.6*</td>
</tr>
<tr>
<td>A\textsubscript{lac}, mmol/L</td>
<td>0.42 ± 0.05</td>
<td>1.05 ± 0.02**</td>
<td>0.52 ± 0.08**</td>
</tr>
<tr>
<td>Hemoglobin, mmol/L</td>
<td>8.83 ± 0.43</td>
<td>8.88 ± 0.36</td>
<td>8.71 ± 0.29</td>
</tr>
<tr>
<td>Mean arterial blood pressure, mm Hg</td>
<td>117 ± 7.0</td>
<td>130 ± 5.0**</td>
<td>121 ± 5.0</td>
</tr>
<tr>
<td>PacO\textsubscript{2}, mm Hg</td>
<td>40.7 ± 6.9</td>
<td>38.3 ± 6.0</td>
<td>38.6 ± 9.3</td>
</tr>
<tr>
<td>(a−v)\textsubscript{O2}, mmol/L</td>
<td>3.23 ± 0.47</td>
<td>3.03 ± 0.32</td>
<td>3.37 ± 0.24</td>
</tr>
<tr>
<td>(a−v)\textsubscript{glc}, mmol/L</td>
<td>0.55 ± 0.11</td>
<td>0.51 ± 0.07</td>
<td>0.53 ± 0.05</td>
</tr>
<tr>
<td>(a−v)\textsubscript{lac}, mmol/L</td>
<td>-0.05 ± 0.02</td>
<td>-0.03 ± 0.06</td>
<td>-0.06 ± 0.01</td>
</tr>
<tr>
<td>OGI</td>
<td>6.2 ± 1.3</td>
<td>6.3 ± 1.3</td>
<td>6.4 ± 0.7</td>
</tr>
<tr>
<td>Movements, min</td>
<td>0.0 ± 0.0</td>
<td>4.2 ± 1.2**</td>
<td>0.1 ± 0.1</td>
</tr>
<tr>
<td>Sniffing, min</td>
<td>2.3 ± 0.6</td>
<td>7.5 ± 1.9**</td>
<td>2.1 ± 0.5</td>
</tr>
</tbody>
</table>

Values are mean ± SD. The values represent the mean of values obtained in 7 animals. These animals were studied during identical conditions, during baseline conditions (Baseline), during activation (Activ.), and in a period after activation was terminated (Postactiv.).

Statistical significance is denoted by *P<0.05; †P<0.01.

Results

Values Obtained Without Administration of Propranolol

Before activation, values for physiological variables and cerebral arteriovenous differences were all within the normal range (Table 1). During activation, the arterial concentrations of glucose (P<0.01) and lactate (P<0.01) increased, whereas PacO\textsubscript{2} was reduced (P<0.05). Activation reduced (a−v)\textsubscript{O2} by 25% (P<0.01), whereas (a−v)\textsubscript{glc} was unchanged. Together, these changes in cerebral arteriovenous differences caused a significant reduction of OGI (34%, P<0.01 (Table 1; Figure 1). The (a−v)\textsubscript{lac} showed a small release from the brain in the control setting, which was reversed to a small uptake during activation (P<0.01), a change that could reflect the increase in the arterial lactate concentration. During the activation phase, all rats showed increased motor activity (P<0.05) and sniffing (P<0.05) (Table 1). Except for the arterial concentration of glucose, which remained increased (P<0.01), all values normalized within the postactivation period (Table 1).

Discussion

In the present study, we present data indicating that the activation-induced uncoupling of CMR\textsubscript{glc} from CMRO\textsubscript{2} by β-adrenergic blockade with propranolol. Such an observation on the brain can be expected at the time of the study, because propranolol had been infused over a period of 1 hour.

Activation Without Propranolol

Data obtained during baseline conditions are almost identical to results that have been obtained in other studies and indicate that our animals were in a nonactivated behavioral state.
oxygen and glucose was 6.1±0.9 (mean±SD) (Table 1). This value signifies that the amount of carbon skeletons that were oxidized or escaping the brain as lactate was slightly larger than the amount of carbon skeletons that was taken up as glucose. This discrepancy is explained by the period of starvation imposed on our rats before the experiment. During starvation, the arterial concentration of ketone bodies increases, and ketone bodies, mainly β-hydroxybutyrate, become an alternative substrate for oxidative metabolism. In a previous study, we determined the cerebral uptake of ketone bodies during conditions identical to the present study (for discussion, see Reference 7). Taking the slight cerebral uptake of ketone bodies that has been observed during identical conditions into account, our data indicate that cerebral carbon uptake during baseline conditions is almost perfectly balanced by cerebral carbon oxygenation and cerebral lactate release.

Without propranolol administration, the data obtained during activation are in close accordance with previous results.7,8 Activation reduced (a−v)O2 by 25% (Table 1). This indicates, as discussed above, that activation increased CBF 33% more than it increased CMRO2 or, less likely, that CMRO2 had decreased. Activation reduced (a−v)O2, whereas (a−v)ck remained essentially unchanged. The flux of lactate expressed as (a−v)lc was reversed from a small efflux from the brain to an uptake corresponding to an increase of the arterial lactate concentration. The molar cerebral uptake ratio between oxygen and glucose, the OGI, was reduced from 6.1 during baseline to 4.0 during activation (Table 1, Figure 1). From the arteriovenous differences we observed during activation, it can be calculated that oxidative metabolism accounted for only 62% of the glucose that was taken up by the brain. The metabolic fate of the activation-induced cerebral glucose accumulation is unknown, and some of the excess glucose uptake may have been converted to lactate that accumulated in the brain. Previous data that have addressed this problem directly, however, indicate that only ~50% of the activation-induced excess glucose uptake can be accounted for by anaerobic conversion to lactate.8

Activation After Administration of Propranolol

In the second part of our study, we activated our animals after administration of propranolol. Propranolol did not influence cerebral arteriovenous differences or physiological variables that were determined before activation (Tables 1 and 2). Values for (a−v)O2, (a−v)ck, and (a−v)lc during baseline conditions were almost identical with and without administration of propranolol. Thus, administration of propranolol totally abolished the uncoupling of CMRO2 from CMRglc and CBF as estimated from the (a−v)O2. With propranolol, all glucose taken up by the brain during activation could be fully accounted for by oxidation and cerebral efflux of lactate.

The lack of activation-induced uncoupling between oxygen and glucose metabolism and CBF was not due to an inadequate activation response in our animals. In our study, we monitored the behavioral degree of activation through assessment of sniffing and body movements in our experimental animals, and in no case did administration of propranolol reduce the behavioral response to activation. Furthermore, the administration of propranolol did not abolish the activation-induced increase in the arterial glucose concentration, and blood pressure during activation was the same with and without propranolol. We therefore conclude that the rats that received propranolol were activated to a degree comparable to those that did not receive any propranolol.

The results of the present study show that β-adrenergic blockade by propranolol abolishes the decrease of the OGI. Thus, β-adrenergic blockade does not only abolish the increase in oxygen consumption and flow during immobilization stress activation, as originally observed by Carlsson et al.9 The mechanisms underlying this effect of propranolol cannot be answered from our study, but it has previously been demonstrated that propranolol blocks the breakdown of cerebral glycogen.16–18 A blockade of an equilibrium between glucose and glycogen in a continuous synthesis and breakdown might be responsible for the lack of decrease of the OGI during activation after propranolol administration.

Conclusions

Our data clearly indicate that administration of propranolol almost totally abolishes the activation-induced uncoupling between CBF, CMRO2, and CMRglc. This observation is important because it may bear significant influence on the subject of the mechanism responsible for the coupling between CBF and cerebral metabolism. It must be emphasized, however, that the present data are obtained in the animal setting and that our data address only global measures of CBF and metabolism. It would be of interest to know whether the effects of propranolol also apply to the regional setting. One easy way to do this would be to determine the effect of β-adrenergic blockade on the activation response as determined by functional MRI. With this technique, the activation-induced change in the amount of reduced hemoglobin is determined regionally. If propranolol abolishes the uncoupling between CBF and CMRO2, it would also be expected to abolish the activation response as detected by functional MRI.

Finally, the observations in the present study raise the important question of how β-adrenergic blockade with propranolol can be tolerated without serious cognitive side effects. If propranolol abolishes the activation-induced changes in the pattern of cerebral metabolism and this does not cause serious cerebral side effects, what is then the purpose of the activation-induced changes in cerebral metabolism?

Acknowledgments

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