EFFECTS OF SUXAMETHONIUM ON THE CEREBRUM FOLLOWING DISRUPTION OF THE BLOOD–BRAIN BARRIER IN DOGS

W. L. LANIER, J. H. MILDE AND F. W. SHARBROUGH

SUMMARY

We have studied the effects of suxamethonium 1.0 mg kg\(^{-1}\) i.v. on cerebral blood flow (CBF), cerebral metabolic rate (CMR\(\text{O}_2\)), and the electroencephalogram (EEG) in dogs anaesthetized with halothane (1.0 MAC) following blood–brain barrier (BBB) disruption with intracarotid (i.c.) mannitol. The combination produced a transient increase in CBF, while CMR\(\text{O}_2\) did not change. These responses were similar to those produced by i.c. mannitol plus i.v. saline. Suxamethonium produced desynchronization of the EEG that persisted longer than that produced by saline. In only one of the six animals was the desynchronization sustained (90 min) beyond that found in dogs with a normal BBB. We conclude that disruption of the BBB did not enhance the cerebral stimulating effects of i.v. suxamethonium, and did not increase the likelihood of seizure activity following suxamethonium.

KEY WORDS


Suxamethonium produces activation of the electroencephalogram (EEG) and increases in cerebral blood flow (CBF) in lightly anaesthetized subjects with an intact blood–brain barrier (BBB) [1]. The response is thought to be caused by a mechanism other than a direct effect on the brain [1, 2], as the drug does not cross the BBB [3]. In contrast, it produces EEG evidence of seizures when placed topically on the cerebral cortex [4]. This suggests that it may be capable of inducing cerebral stimulation or seizures in patients with impairment of the BBB. The present study tested this hypothesis by administering i.v. suxamethonium to dogs following osmotic disruption of the BBB.

MATERIALS AND METHODS

The programme for this study was approved by the Institutional Animal Care and Use Committee. Anaesthesia was induced and maintained with 1–3 % halothane and nitrogen in oxygen in 12 unpremedicated mongrel dogs weighing 8.0–14.1 kg. The trachea was intubated without the aid of neuromuscular blockers and the lungs were ventilated with a Harvard ventilator. Ventilation and \(F_{\text{I}O_2}\) were adjusted to maintain \(P_{\text{A}O_2}\) and \(P_{\text{ACO}_2}\) close to 20.0 kPa and 5.3 kPa, respectively. Cannulae were inserted into a femoral artery for blood sampling and pressure measurements, and into femoral and forelimb veins for administration of fluids and drugs. Teflon cannulae (20-gauge, 32 mm) were inserted into the common carotid arteries bilaterally, with their tips at the junction of the external and internal carotid arteries, for the injection of mannitol. Heart rate (HR) was measured from a lead II electrocardiogram. During the preparatory period, dogs were given 0.9 % saline solution

WILLIAM L. LANIER, M.D., JAMES H. MILDE (Department of Anesthesiology); FRANK W. SHARBROUGH, M.D. (Department of Neurology); Mayo Clinic and Mayo Medical School, 200 First Street, SW, Rochester, Minnesota 55905, U.S.A. Accepted for Publication: June 11, 1990.
20 ml kg⁻¹ i.v. Sodium bicarbonate was given i.v. as needed to maintain a buffer base of approximately 40 mmol litre⁻¹.

After heparinization with 300–400 u kg⁻¹ i.v., the sagittal sinus was exposed, isolated and cannulated as described previously [5]. This allowed blood sampling and provided direct measurement of CBF from the anterior, superior and lateral portions of both cerebral hemispheres, representing approximately 54% of the total brain weight [6]. Blood flow was recorded continuously using a square wave electromagnetic flow meter (ET 300 API, Carolina Medical Electronics) [7]. Blood oxygen contents were calculated from measurements of oxyhaemoglobin concentrations (CO-oximeter, IL 282) and oxygen tensions (IL electrodes) [8]. CMRO₂ was calculated as the product of CBF and the arterial–sagittal sinus blood oxygen content difference. A six-lead, three-channel bipolar (bifrontal, biparietal and bioccipital) EEG was recorded from electrodes glued to the calvarium. Brain temperature was monitored by a parietal extradural thermistor and maintained at 37 °C with heat lamps. Inspired and end-expired concentrations of halothane and carbon dioxide were measured with a mass spectrometer (Perkin-Elmer Model 1100). Serum osmolality was determined by the freezing point depression technique (Multi-Osmette, Precision Systems, Inc.). The ears of all dogs were plugged with cotton and their eyes were closed with tape.

Halothane concentrations were maintained at 0.87% end-expired (1.0 MAC) for 20 min before control measurements were taken. During the 20-min stabilization period, no additional i.v. fluids were given and ventilation and F₁O₂ were unchanged. After control measurements were taken, dogs were assigned to one of two treatment groups: six dogs (group A) received intracarotid (i.c.) injections of mannitol, followed 3.0 min later by i.v. suxamethonium 1.0 mg kg⁻¹ in 0.9% saline 3.0 ml; an additional six dogs (group B) received i.c. mannitol followed 3.0 min later by i.v. 0.9% saline 3.0 ml. The first dog in each of these groups received 25% mannitol 2.0 ml kg⁻¹ into each carotid artery over 40 s (total dose 1.0 g kg⁻¹). This dose produced less than the desired amount of BBB disruption of the frontal cortex, so the remaining five dogs in each group received 25% mannitol 3.0 ml kg⁻¹ into each carotid artery injected over 20 s (total 1.5 g kg⁻¹).

The combination of i.c. mannitol plus i.v. suxamethonium produced a mixed metabolic and respiratory acidosis and ventilation was increased by 30% concomitantly with administration of neuromuscular blocker in order to keep end-tidal carbon dioxide stable in those dogs. Following the 15-min measurement point in both groups of dogs, bicarbonate was given and ventilation was adjusted in an attempt to restore acid–base status to near control values.

Following the 15-min measurement point, all dogs were given i.v. infusions of 3% Evans blue dye 4 ml kg⁻¹ over a 5-min period for determination of the integrity of the BBB [9]. Administration of this hypertonic solution, and the prior administration of mannitol i.e., produced a marked decrease in mean arterial pressure [10] which was countered with an infusion of phenylephrine 40 µg ml⁻¹ in 0.9% saline solution during infusion of mannitol and Evans blue dye to maintain cerebral perfusion pressure > 50 mm Hg.

At the completion of the study, the dogs were killed with potassium chloride i.v. The brains were flushed with 0.9% saline solution through the carotid artery cannulae until the sagittal sinus effluent was clear. The brains were removed and weighed, rinsed with saline solution and stored in 3.7% buffered formalin solution. After a minimum of 4 weeks’ storage, they were sliced in the coronal plane at five different sites between the frontal pole and the site of sagittal sinus cannulation. The degree of BBB disruption for each slice was scored according to a four-point scale [11]. The degree of BBB disruption for a given dog was defined as the median response of the various slices.

Following treatment with either suxamethonium or saline, data were grouped into 15-min measurement periods. For each measurement period, the mean response during the period was calculated as the integral of the response divided by time. Such an analysis reduced the number of comparisons within each group and between groups. The individual data points following either suxamethonium or saline treatment, in addition to data from the 15-min measurement intervals, were compared with the appropriate control data using a one-factor analysis of variance and F tests. Data from groups given suxamethonium were compared with those from groups receiving saline placebo, using Student’s unpaired t tests. Data evaluating the degree of BBB staining were compared using the Mann–Whitney rank sum test. P < 0.05 was considered significant.
TABLE I. Control variables (mean (SEM)) before blood–brain barrier disruption in dogs treated with either suxamethonium or placebo. CBF = Cerebral blood flow; CMRO₂ = cerebral metabolic rate; MAP = mean arterial pressure

<table>
<thead>
<tr>
<th></th>
<th>Group A</th>
<th>Group B</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Suxamethonium 1.0 mg kg⁻¹ i.v.</td>
<td>Placebo i.v.</td>
</tr>
<tr>
<td>(n = 6)</td>
<td></td>
<td>(n = 6)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>10.8 (0.7)</td>
<td>10.6 (0.7)</td>
</tr>
<tr>
<td>Pao₂ (kPa)</td>
<td>20.1 (0.4)</td>
<td>20.1 (0.3)</td>
</tr>
<tr>
<td>Pco₂ (kPa)</td>
<td>5.2 (0.1)</td>
<td>5.1 (0.1)</td>
</tr>
<tr>
<td>pH</td>
<td>7.33 (0.01)</td>
<td>7.34 (0.02)</td>
</tr>
<tr>
<td>Buffer base (mmol litre⁻¹)</td>
<td>41 (1)</td>
<td>41 (1)</td>
</tr>
<tr>
<td>Haemoglobin (g dl⁻¹)</td>
<td>13.0 (0.7)</td>
<td>12.3 (0.8)</td>
</tr>
<tr>
<td>Serum osmolality (mosmol litre⁻¹)</td>
<td>295 (1)</td>
<td>296 (2)</td>
</tr>
<tr>
<td>CBF (ml 100 g⁻¹ min⁻¹)</td>
<td>67 (5)</td>
<td>70 (6)</td>
</tr>
<tr>
<td>CMRO₂ (ml 100 g⁻¹ min⁻¹)</td>
<td>3.64 (0.12)</td>
<td>3.88 (0.17)</td>
</tr>
<tr>
<td>MAP (mm Hg)</td>
<td>100 (3)</td>
<td>97 (4)</td>
</tr>
<tr>
<td>Brain temperature (°C)</td>
<td>37.1 (0.0)</td>
<td>37.1 (0.1)</td>
</tr>
<tr>
<td>Oesophageal temperature (°C)</td>
<td>37.2 (0.1)</td>
<td>37.1 (0.1)</td>
</tr>
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</table>

RESULTS

Control systemic and cerebral variables measured immediately before BBB disruption are listed in table I. There were no significant differences between groups. Postmortem examination of the formalin-preserved brains demonstrated intraparenchymal Evans blue dye throughout the cortex, denoting BBB disruption. All slices in all dogs had some evidence of BBB disruption, and there was no significant difference between the amount of BBB disruption in the two experimental groups.

Mannitol i.e. produced EEG activation in all dogs; however, there was a difference between the EEG effect of mannitol plus suxamethonium (group A) and that of mannitol plus placebo (group B). In both groups, the control EEG consisted predominantly of an alpha rhythm with a superimposed delta rhythm. The alpha component was typically 8–10 Hz and 25–100 μV; the delta component was 2–4 Hz and 50–300 μV. In both groups, i.e. mannitol produced immediate desynchronization of the EEG. In one group B dog there was a 30-s period of mild desynchronization that was not typical of the degree or duration seen in the other 11 dogs. The activation in these 11 dogs was an increase in the frequency of the alpha rhythm to 12–14 Hz, with a reduction in the alpha amplitude of 30–50%. There was also a concomitant elimination or attenuation of the delta component of > 80%. In the five group B dogs showing EEG activation, the EEG returned to a synchronized pattern similar to that recorded in the control period (within 2–3 min in four, and within 4 min in one). The manner in which the EEG returned to a synchronized pattern in group B differed from that observed in group A: in the latter group, one dog had EEG desynchronization that persisted from the administration of mannitol to the 90-min measurement period. In an additional three dogs, the EEG remained desynchronized from the administration of mannitol until 3 min after suxamethonium. In the remaining two group A dogs, the mannitol-induced EEG changes returned to control values at 1.5 and 2.5 min after i.e. mannitol. Subsequent administration of suxamethonium produced a further period of desynchronization in both, similar to that observed following i.e. mannitol. The desynchronization persisted for 1.0 and 2.0 min, respectively.

EEG evidence of seizure activity was not observed in any animal. Neither mannitol plus suxamethonium in group A nor mannitol plus placebo in group B had an effect on CMRO₂. There were no differences in CMRO₂ between the two groups (fig. 1).

In group A, mannitol plus suxamethonium produced a significant increase in CBF from a control value of 67 (SEM 5) ml 100 g⁻¹ min⁻¹ to a peak value of 117 (9) ml 100 g⁻¹ min⁻¹ (a 75% increase from control) at the 1-min measurement point. This was followed by a decline in CBF toward control values with time. This effect on CBF was similar to the effect of mannitol plus saline in group B (fig. 1). In group B, the control CBF was 70 (6) ml 100 g⁻¹ min⁻¹. Injection of
BLOOD–BRAIN BARRIER AND SUXAMETHONIUM

CO 2 

Time (min)

FIG. 1. CBF, CMRO₂ and MAP following suxamethonium (●) or placebo (○) treatment. Each point represents the mean response for six dogs. No significant differences between groups. C = Control period immediately before injection of mannitol. * Blood oxygen content, and thus CMRO₂, could not be measured spectrophotometrically after i.v. Evans blue dye.

mannitol plus i.v. saline produced a transient cerebral hyperaemia to a peak value of 122 (20) ml 100 g⁻¹ min⁻¹ (a 74 % increase from control) at the 1-min measurement period, and this was followed by a decline toward control values.

When the mean CBF during the 15-min intervals was compared with the control CBF, the 0–15 min CBF value increased significantly in group A. In this group, the mean 0–15 min value was 92 (8) ml 100 g⁻¹ min⁻¹ (a 37 % increase from control; P = 0.02 vs control). Although the CBF data over the same interval in group B did not achieve statistical significance at the P < 0.05 level, there was a tendency to increased flow. In group B, the mean 0–15 min CBF value was 90 (13) ml 100 g⁻¹ min⁻¹ (a 28 % increase from control; P = 0.08). When the mean CBF data from group A and group B were compared with each other, there were no significant differences at any measurement interval.

There was no significant change in MAP with time in group A. In contrast, in group B, there was an initial increase in MAP (probably related to an overzealous administration of phenylephrine) that was significantly different from control values at the 0–15 and 15–30 min measurement periods. However, there were no significant differences between groups at any measurement period, although there was a tendency to a greater MAP in group B at the 0–15 min period (P = 0.08).

There was a tendency for increases in PaCO₂ after administration of mannitol and the study treatment in both groups A and B; however, in neither group did the values differ significantly from control, neither did the values differ between groups A and B.

DISCUSSION

When i.v. suxamethonium is administered to lightly anaesthetized subjects with normal BBB, the result is a brief period of EEG activation [1, 2] and increases in CBF [1, 2] and intracranial pressure [1, 12]. It is unlikely that the cerebral response is caused by direct interaction between the agent and cerebral neurones, because suxamethonium does not cross the BBB [3], and it has no effect on cerebral function when injected into the carotid arteries [13] or when given i.v. in subjects with spinal cord transection [13]. Instead of a direct neuronal effect, cerebral stimulation by suxamethonium has been attributed primarily to a modulation of cerebral function resulting from increased afferent input into the brain from muscle stretch receptors (increases in muscle afferent activity) [1, 2, 13].

Although i.v. suxamethonium can produce cerebral function alterations without crossing the BBB, there is some evidence to suggest that the direct contact between suxamethonium and cerebral neurones results in a more pronounced cerebral effect. Tan reported that topical application of suxamethonium to the brain resulted in EEG evidence of seizures [4]. Although suxamethonium has been administered to many patients with brain injury and disrupted BBB, the production of seizures by the drug in this context has never been recognized as a likely clinical phenomenon. However, this is confused by the fact that suxamethonium produces neuromuscular paralysis, thus the motor manifestations of any early seizure activity would be prevented, and suxamethonium is given usually with anaesthetic drugs, many of which are profound anticonvulsants (e.g. thiopentone). Finally, the presence of seizures in brain-injured subjects would probably be attributed to the brain injury, and not to a drug effect. The present study was designed to elim-
inate many of these confounding variables. Despite the study design, no convulsant effect of suxamethonium was demonstrated.

There are several factors that may explain the apparent discrepancy between the topical cortical effects and the absence of seizure activity when the myoneural blocker was given i.v. in the presence of disrupted BBB. First, direct application of suxamethonium to the cerebral cortex results in neuronal concentrations in excess of those obtained following the i.v. administration of suxamethonium in the presence of disrupted BBB. Second, it is possible that our methods did not allow suxamethonium to pass the BBB. We did not measure the passage of suxamethonium across the BBB, but assumed that BBB disruption sufficient to allow the passage of Evans blue dye (charged; anhydrous molecular weight 961 Da before binding with proteins; molecular weight of dye–albumin complex approximately 68 500 Da [9]) would also permit the passage of suxamethonium (charged; anhydrous molecular weight 361 Da; hydrated molecular weight 397 Da [14]). Thus direct passage of suxamethonium across the BBB was likely to have occurred.

In summary, the administration of clinically relevant doses of i.v. suxamethonium in the presence of osmotically disrupted BBB produced minimal alterations of cerebral function other than those attributed to BBB disruption alone. Specifically, no EEG, CBF or CMRO₂ evidence of intense cerebral stimulation or seizures was noted. We conclude that disruption of the BBB is not a contraindication to the use of i.v. suxamethonium.

ACKNOWLEDGEMENTS

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REFERENCES