ELECTROENCEPHALOGRAPHIC EFFECTS OF LAUDANOSINE IN AN ANIMAL MODEL OF EPILEPSY

A. TATEISHI, M. H. ZORNOW, M. S. SCHELLER AND P. C. CANFELL

Atracurium is a non-depolarizing neuromuscular blocking agent of intermediate duration which is used widely during surgical procedures for which muscle paralysis is indicated. Atracurium is not only metabolized by ester hydrolysis, but also degrades spontaneously in the body by Hofmann elimination at physiological pH and temperature [1]. Because the offset of the action of this drug is independent of liver or kidney function, atracurium may be the preferred neuromuscular blocking agent for patients with hepatic or renal insufficiency. One of the principal metabolites of atracurium, laudanosine \([3',4'-\text{dimethoxy benzyl}]2\)-methyl-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline\), is a known central nervous system (CNS) stimulant [2, 3]. Laudanosine has been shown to cause seizures in animals only at plasma concentrations which greatly exceed those expected following the routine clinical use of atracurium [4]. It is not known, however, if clinically relevant concentrations of laudanosine could provoke seizure activity in patients with pre-existing cerebral pathology (brain tumour, trauma, epileptogenic focus). We therefore examined electroencephalographic effects of laudanosine in an animal model of induced epilepsy.

MATERIALS AND METHODS

Fourteen New Zealand White rabbits weighing 2.5–3.9 kg were studied following approval by the Animal Care Committee. They were initially anaesthetized with \(4^o_0\) halothane in oxygen. Following paralysis with pancuronium 0.3 mg kg\(^{-1}\) i.v. the trachea was intubated orally and the lungs ventilated mechanically with 30\(^o_0\) oxygen and 1.0–1.5\(^o_0\) halothane in nitrogen. Tidal volume and rate of ventilation were adjusted to 15 ml kg\(^{-1}\) and 30 b.p.m., respectively. The \(P_{a,o_2}\) was maintained at 4.7–6.0 kPa through the addition of carbon dioxide to the inspired gas mixture. Oeso-

SUMMARY

We examined the effects of laudanosine, one of the principal metabolites of atracurium, on the electroencephalogram (EEG) in an animal model of induced epilepsy. Fourteen rabbits were anaesthetized with 4\(^o_0\) halothane in oxygen, the trachea intubated and the lungs ventilated mechanically with 30\(^o_0\) oxygen and 1\(^o_0\) halothane in nitrogen. Animals were assigned randomly to receive either an infusion of laudanosine (laudanosine group, \(n = 7\)) at a rate calculated to produce plasma concentrations similar to those found following the clinical use of atracurium, or an equal volume of normal saline (control group, \(n = 7\)). To induce an epileptogenic focus, gel foam sponges soaked in a pH-adjusted 4\(^o_0\) cefazolin solution were applied bilaterally to the parietal cortical surface. This resulted in the production of spike and burst EEG activity in all animals. However, scoring the frequency of the spikes and bursts revealed no significant differences between the laudanosine and control groups. We conclude that, in this animal model of epilepsy, no increased incidence of seizure activity was produced by mean plasma laudanosine concentrations as great as 0.8 \(\mu g \text{ ml}^{-1}\). These results suggest that the routine use of atracurium is unlikely to provoke seizures, even in the presence of an epileptogenic focus.
Pharyngeal temperature was maintained at 37.5 °C through the use of a servo-controlled infra-red lamp and a heating pad. Catheters were inserted into the femoral artery and vein for the continuous monitoring of arterial pressure, intermittent sampling of arterial blood, and for the continuous infusion of normal saline 4 ml kg⁻¹ h⁻¹ and pancuronium 0.5 mg h⁻¹. The animals were then placed in the "sphinx" position and the head fixed in a stereotaxic frame. The scalp was incised in the midline and muscles were reflected laterally to expose the skull. All the incision sites were infiltrated subcutaneously with 0.25% bupivacaine. Brass screw electrodes were placed bilaterally in the skull over the frontal and occipital regions for the extradural recording of the cortical electroencephalogram (EEG) (Model 78, Grass Instrument, Quincy, MA). Biparietal craniectomies (approximately 4 mm in diameter) were made for the later application to the cortical surface of gelfoam sponges. The dural membrane under each craniectomy was carefully incised with a surgical blade to avoid injury to the underlying cortex.

Following the completion of the surgical preparation, the end-tidal halothane concentration was decreased to 1.0%, and maintained at this value for at least 10 min before the control EEG recordings (C1) were made. The animals were then assigned randomly to receive either a loading infusion of laudanosine (Aldrich Chemical Co., Milwaukee, WI) at 96 ng kg⁻¹ min⁻¹ (laudanosine group) or an equal volume of normal saline (control group) for a period of 10 min. The EEG was again recorded (C2) and 4% cefazolin-soaked sponges (4 mm diameter, 1 mm thick) were applied bilaterally to the cortical surface. The pH of cefazolin solution was titrated to 7.20–7.30 with NaOH 0.1 mol litre⁻¹. The rate of infusion of laudanosine (or the same volume of normal saline) was simultaneously decreased to 24 μg kg⁻¹ min⁻¹, and continued for the remainder of the experiment. Based upon the pharmacokinetic data of Shi and colleagues [2], this infusion regimen was expected to produce plasma laudanosine concentrations of approximately 0.8 μg ml⁻¹. Repeated recordings of the EEG and blood-gas analyses were made at 5, 10, 20, 30, 45 and 60 min following application of the cefazolin. Plasma samples were obtained at 20 and 60 min after the application of cefazolin from four laudanosine animals for the determination of laudanosine concentration by ion-exchange liquid chromatography [5]. Arterial blood-gas tensions and pH were measured by appropriately calibrated electrodes at 37 °C (IL-813, Instrumentation Laboratory, Lexington, MA). EEG were subsequently scored in a blinded fashion for the frequency of spike and burst activity. Spikes were defined as sharp wave activities with an amplitude greater than or equal to twice that of the background EEG. Bursts were defined as a continuous run of three or more spike waves.

Changes in physiological variables were examined within each group using a repeated measures analysis of variance, while comparisons between groups were examined with unpaired t tests. EEG scores were examined using a two-factor repeated measures analysis of variance. In addition, mean values for spike and burst frequency over time were calculated for each animal and intergroup comparisons were made using an unpaired two-tailed t test. Significance was assumed at P < 0.05.

**RESULTS**

There were no significant differences between or within the two groups in terms of arterial pressure, blood-gas values (table I), or oesophageal temperature at any point during the experiment. In both groups, spike activity developed within 5 min after cefazolin sponges were applied to the

**Table I. Mean (SD) values of physiological variables obtained before and 60 min after application of cefazolin. There were no differences between groups**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control</th>
<th>Laudanosine</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAP (mm Hg)</td>
<td>79 (7)</td>
<td>81 (5)</td>
</tr>
<tr>
<td>pH</td>
<td>7.41 (0.04)</td>
<td>7.41 (0.04)</td>
</tr>
<tr>
<td>$P_aO_2$ (kPa)</td>
<td>5.3 (0.5)</td>
<td>5.3 (0.4)</td>
</tr>
<tr>
<td>$P_aCO_2$ (kPa)</td>
<td>24.3 (1.3)</td>
<td>22.7 (2.7)</td>
</tr>
</tbody>
</table>
Infusion of laudanosine or saline

Fig. 1. Mean (SEM) frequency of spike activity in the EEG of control (□) and laudanosine (■) groups. Arrow indicates the application of cefazolin (CFZ) to the cortex. Laudanosine or the same volume of normal saline were infused at a rapid (96 µg kg⁻¹ min⁻¹) and then a slow rate (24 µg kg⁻¹ min⁻¹). Cortex, and continued throughout the experiment. The frequency of burst activity increased and peaked between 10 and 30 min in both group (figs 1, 2). However, there were no significant differences between the control and laudanosine groups in spike or burst frequency at any time point during the experiment, as revealed by analysis of variance. Analysis of mean spike and burst frequency showed no differences between the two groups (P = 0.73 and 0.09, respectively). Figure 3 shows a representative EEG pattern after the application of cefazolin. The infusion of laudanosine resulted in a mean plasma concentration of 0.83 (0.09) µg ml⁻¹.

DISCUSSION

Laudanosine has a long elimination half-life (T₁/₂ = 200 min in man), and easily penetrates the blood–brain barrier [1, 6, 7]. The peak concentration of laudanosine in the cerebrospinal fluid was found to occur 5–10 min after a bolus

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**Fig. 2.** Mean (SEM) frequency of burst activity in the EEG of control (□) and laudanosine (■) groups. Arrow indicates the application of cefazolin (CFZ) to the cortex. Laudanosine or the same volume of normal saline were infused at a rapid (96 µg kg⁻¹ min⁻¹) and then a slow rate (24 µg kg⁻¹ min⁻¹).

**Fig. 3.** Representative EEG changes induced by cefazolin. A: Spike wave activity 10 min after cortical application of cefazolin. B: Burst activity 20 min after cefazolin.
LAUDANOSINE AND SEIZURES

Injection, and varied between 36% and 87% of the corresponding plasma concentration [8]. In sufficient doses, laudanosine has been reported to produce convulsive movements or seizure activity on the EEG in a number of animal species [4, 9, 10]. An infusion of laudanosine 100 mg kg⁻¹ h⁻¹ caused convulsions in five awake rats (cumulative dose varied between 38.2 and 94.4 mg kg⁻¹), with the corresponding plasma laudanosine concentrations being greater than 20 μg ml⁻¹ [4]. During the continuous infusion of laudanosine to halothane-anaesthetized dogs, a laudanosine concentration greater than 10 μg ml⁻¹ induced EEG spikes, while concentrations greater than 17 μg ml⁻¹ produced prolonged seizures (at cumulative doses of 30-50 mg kg⁻¹) [9]. Smaller doses (14-22 mg kg⁻¹) were reported to produce seizure activity on EEG in hyperventilated dogs [8]. In the majority of animal studies, the epileptogenic plasma concentration of laudanosine was at least eight times greater than that seen following the intraoperative use of atracurium. Even after 5-6 days of continuous neuromuscular block produced by an atracurium infusion to patients undergoing mechanical ventilation in an intensive care unit, the peak plasma concentration of laudanosine was 5 μg ml⁻¹ and there was no evidence of cerebral excitation [11]. Renal function may have some role in excretion of unchanged laudanosine. Peak laudanosine concentrations after a single 0.5-mg kg⁻¹ dose of atracurium were reported to be 0.327 and 0.758 μg ml⁻¹ in normal and renal failure patients, respectively, although this difference was not statistically significant [12]. These concentrations are considerably lower than those reported with CNS excitation. However, despite these seemingly reassuring statistics, a question has remained as to whether or not patients with cerebral pathology are more sensitive to the epileptogenic effects of laudanosine.

To test this possibility, we assessed the EEG effects of clinically relevant concentrations of laudanosine in an established model of epilepsy. Laudanosine was infused in a manner which produced plasma concentrations of 0.8 μg ml⁻¹. This concentration may be seen following the routine intraoperative use of atracurium in patients with renal failure. The cerebral application of cephalosporin compounds has been shown to produce the behavioural and electrophysiological patterns of epilepsy [13]. The close relationship between the chemical structure of the cephalosporins and their epileptogenic activity has led to the assumption that those compounds promote the mechanisms of convulsive discharge of the neuronal cell membrane [14]. Cefazolin is known to possess potent epileptogenic properties and 2% and 4% solutions have been used to evaluate the anti-convulsant potential of the calcium channel blocker, nimodipine [15], the 4% solution producing a more intense epileptic activity than 2%. We used the more epileptogenic 4% concentration, and found that cortically applied cefazolin clearly produced EEG spikes and bursts. However, the frequencies of spikes and bursts were not significantly different between groups at any time, suggesting that laudanosine did not enhance seizure activity in this model. There was no apparent trend towards a difference between the groups in terms of spike frequency. Inspection of the burst data reveals that the mean number of bursts was slightly greater in laudanosine group at all times, although statistical significance could not be demonstrated at the 0.05 level of probability. Even if a statistical difference existed between groups in terms of burst frequency which was not apparent because of the relatively large standard errors in this study (type II error), the magnitude of the difference is not likely to have clinical importance. The mean number of bursts in the laudanosine group was greater by only approximately 2 min⁻¹ compared with the control group. Sustained seizure activity was not observed in any of the laudanosine or control animals.

To our knowledge, these are the only data examining the propensity of laudanosine to activate epileptic activity in other than normal animals. These data, together with absence of case reports to the contrary despite the widespread usage of atracurium, cast doubt on the epileptogenic potential of clinically relevant plasma concentrations of laudanosine in the presence of excitable brain pathology.

In summary, in a topical antibiotic-induced epilepsy model, no increased incidence of seizure activity was detected in rabbits receiving laudanosine at rates sufficient to produce plasma concentrations similar to those seen following the clinical use of atracurium.

REFERENCES


