



SCIENTIFIC ARTICLE

The effects of intra-cerebroventricular administered rocuronium on the central nervous system of rats and determination of its epileptic seizure-inducing dose



Mehmet Baykal^a, Necati Gökmen^a, Alper Doğan^a, Serhat Erbayraktar^b, Osman Yılmaz^c, Elvan Ocmen^a, Hale Aksu Erdost^{a,*}, Atalay Arkan^a

^a Dokuz Eylul University, Department of Anesthesiology, Izmir, Turkey

^b Dokuz Eylul University, Department of Neurosurgery, Izmir, Turkey

^c Dokuz Eylul University, Department of Animal Research Center, Izmir, Turkey

Received 12 December 2014; accepted 23 February 2015

Available online 19 June 2016

KEYWORDS

Rocuronium;
Seizure;
Central nervous system;
Rat

Abstract

Background: The aim of this study was to investigate the effects of intracerebroventricularly administered rocuronium bromide on the central nervous system, determine the seizure threshold dose of rocuronium bromide in rats, and investigate the effects of rocuronium on the central nervous system at 1/5, 1/10, and 1/100 dilutions of the determined seizure threshold dose.

Methods: A permanent cannula was placed in the lateral cerebral ventricle of the animals. The study was designed in two phases. In the first phase, the seizure threshold dose of rocuronium bromide was determined. In the second phase, Group R 1/5 ($n=6$), Group 1/10 ($n=6$), and Group 1/100 ($n=6$) were formed using doses of 1/5, 1/10, and 1/100, respectively, of the obtained rocuronium bromide seizure threshold dose.

Results: The rocuronium bromide seizure threshold value was found to be $0.056 \pm 0.009 \mu\text{mol}$. The seizure threshold, as a function of the body weight of rats, was calculated as $0.286 \mu\text{mol/kg}^{-1}$. A dose of 1/5 of the seizure threshold dose primarily caused splayed limbs, posturing, and tremors of the entire body, whereas the dose of 1/10 of the seizure threshold dose caused agitation and shivering. A dose of 1/100 of the seizure threshold dose was associated with decreased locomotor activity.

Conclusions: This study showed that rocuronium bromide has dose-related deleterious effects on the central nervous system and can produce dose-dependent excitatory effects and seizures. Published by Elsevier Editora Ltda. on behalf of Sociedade Brasileira de Anestesiologia. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

* Corresponding author.

E-mail: hale.erdost@deu.edu.tr (H.A. Erdost).

<http://dx.doi.org/10.1016/j.bjane.2015.02.010>

0104-0014/Published by Elsevier Editora Ltda. on behalf of Sociedade Brasileira de Anestesiologia. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

PALAVRAS-CHAVE

Rocurônio;
Convulsão;
Sistema nervoso
central;
Rato

Efeitos da administração intracerebroventricular de rocurônio sobre o sistema nervoso central de ratos e determinação da dose indutora de crise epiléptica**Resumo**

Justificativa: O objetivo deste estudo foi investigar os efeitos do brometo de rocurônio administrado intracerebroventricularmente sobre o sistema nervoso central, determinar a dose do limiar convulsivo de rocurônio em ratos e investigar os efeitos de rocurônio no sistema nervoso central em diluições de 1/5, 1/10 e 1/100 da dose do limiar convulsivo determinada.

Métodos: Uma cânula permanente foi colocada no ventrículo lateral do cérebro dos animais. O estudo foi projetado em duas fases. Na primeira fase, a dose do limiar convulsivo do brometo de rocurônio foi determinada. Na segunda fase, o Grupo R 1/5 (n=6), Grupo 1/10 (n=6) e Grupo 1/100 (n=6) foram formados usando doses de 1/5, 1/10 e 1/100, respectivamente, da dose do limiar convulsivo de brometo de rocurônio obtida.

Resultados: Descobrimos que o valor do limiar convulsivo de brometo de rocurônio é $0,056 \pm 0,009 \mu\text{mol}$. O limiar convulsivo, como uma função do peso corporal dos ratos, foi calculado como $0,286 \mu\text{mol}/\text{kg}^{-1}$. Uma dose de 1/5 da dose do limiar convulsivo causou principalmente abertura postural dos membros e tremores em todo o corpo, enquanto uma dose de 1/10 da dose do limiar convulsivo causou agitação e tremores. Uma dose de 1/100 da dose do limiar convulsivo foi associada à diminuição da atividade locomotora.

Conclusões: Este estudo mostrou que o brometo de rocurônio possui efeitos deletérios relacionados com a dose sobre o sistema nervoso central e pode produzir efeitos excitatórios dependentes da dose e convulsões.

Publicado por Elsevier Editora Ltda. em nome de Sociedade Brasileira de Anestesiologia. Este é um artigo Open Access sob uma licença CC BY-NC-ND (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

Introduction

In cases in which neuromuscular blocking drugs have been administered accidentally into the cerebrospinal fluid (CSF),¹ myotonia, autonomic changes, and convulsions have occurred.² Prolonged administration of rocuronium in critical care patients has resulted in the drug entering the CSF.³ Also in patients with impaired function of the blood brain barrier these molecules may reach the CSF. Tassonyi et al.⁴ has reported that some neuromuscular agents like atracurium and its metabolite laudanosine present in the CSF, vecuronium could not be detected in CSF in the subarachnoid hemorrhage. And also atracurium persisted in the CSF for several hours after the end of the infusion. Although it is a non-depolarizing, ionized medication with relatively low lipophilicity, it is known that rocuronium can permeate the CSF after intravenous injection.⁵ Accidental administration of rocuronium into the central nervous system (CNS) during regional anesthesia has been reported.⁶ There are limited data regarding the effects of these drugs on the CNS.

The aim of this study was to investigate the effects of intracerebroventricularly administered rocuronium bromide on the CNS, determine the seizure threshold dose of rocuronium bromide in rats, and investigate the effects of rocuronium on the CNS at 1/5, 1/10, and 1/100 dilutions of the seizure threshold dose.

Methods

The study protocol was approved by the Animal Research Committee of Dokuz Eylul University. Thirty-six female

Wistar albino rats that were bred in the Experimental Research Laboratory of Dokuz Eylul University were used in the study. The rats weighed 180–260 g and were 87% homogeneous, were aged 18–24 months, and exhibited normal activity. The animals were kept under standard laboratory conditions for one week before the experiments began (12 h light: 12 h light, 20–22 °C room temperature) to adapt to the environment. They had free access to water and pellet food, and the study was conducted in a noise-attenuated environment and at the same time each day.

Surgery

Anesthesia was induced by intraperitoneal injection of $50 \text{ mg}/\text{kg}^{-1}$ sodium thiopental (Pental® Sodyum I.E. Ulugay Ilaç San. TAS, Istanbul, Turkey) while maintaining spontaneous respiration and the blink reflex. The rats were placed in the prone position, and a $4 \text{ cm} \times 2 \text{ cm}$ area between the ears and extending toward the nasus was shaved. The area was cleaned with povidon iodine, 1 mL of sterile 1% lidocaine (Aritmal® 2% amp, Biosel Ilaç Sanayi ve Ticaret A.S, Istanbul, Turkey) was infiltrated under the scalp, and a midline surgical incision was made. The head of the animal was placed in a stereotaxic frame where the head was secured in a flexed-forward position. The skin was laterally reflected, and the exposed cranium was gently blunt-dissected with the bregma exposed. A 2 mm hole for the intracerebroventricular rocuronium bromide injection was bored at the stereotaxic coordinates, determined according to the atlas of Paxinos and Watson,⁷ to access the lateral ventricle. The cannula used for the intracerebroventricular procedures

(C311/G, 20G, Plastics One Inc., VA, USA) and a suitable stylet (C311/DC, Plastics One Inc., VA, USA) to prevent tissue and/or foreign bodies from moving into the cannula were placed. The placement of the cannula in the lateral ventricle was confirmed by CSF drainage through the cannula. The edges of the incision were approximated, sutured with 2/0 silk thread, and cleaned with 10% povidon iodine. These procedures were conducted under sterile conditions. Forty-eight hours were allowed for the usual activity of the rats to be restored after this intervention. The purpose of placing a permanent cannula was to enable ICV drug administration while the rats were awake and mobile.

Preparation of the drug

A stock solution with 0.016 μmol rocuronium bromide (Esmeron[®], Organon Corp, Oss, Holland) in 10 μL was used to determine the seizure threshold dose of rocuronium bromide.

Following the determination of the seizure threshold dose of rocuronium, the 1/5, 1/10, and 1/100 dilutions in 10 μL Ringer's lactate solution (Ringer lactate, Biosel Ilac San. TAS, Istanbul, Turkey) were prepared.

The pH values of the rocuronium bromide, Ringer's lactate, and rocuronium bromide-Ringer's lactate solutions at 23 °C were measured with a pH meter (InoLab[®] 720, WTW Wissenschaftlich-Technische Werkstätten GmbH, Munich, Germany).

Experimental protocol

Determination of the seizure threshold dose of rocuronium bromide (Pilot study): To determine the seizure threshold dose of rocuronium bromide, a total of 12 rats were randomized into 2 groups.

Group rocuronium (n=6): To determine the seizure threshold dose, a vinyl-coated polyethylene adapter was attached to the ICV cannula and a Hamilton micro-syringe was attached to this adapter (Hamilton[®] 710SNR 100 μL Syringe Hamilton 710 series syringe, NV, USA). Rocuronium bromide (0.016 $\mu\text{mol} \times 10 \mu\text{L}^{-1}$, total 0.08 μmol) was injected via the Hamilton syringe in divided doses of 5 μL . Each dose was administered consecutively over 60 s, during which time the effects of the dose on the rats were observed. The total dose required to induce a tonic-clonic seizure was recorded.

Group control (n=6): A total of 50 μL of Ringer's lactate solution, in divided doses of 5 μL , was administered via the ICV cannula, as explained above. The rats that did not have seizures were sacrificed after a 6 h observation period.

Dose-response study groups (experimental study): After the determination of the seizure threshold dose of rocuronium bromide, the rats were randomized into 4 groups for the dose-response study.

Group 1 (Group C) (n=6): Ten microliters of a mixture of Ringer's lactate solution and acetic acid with a pH identical to that of the rocuronium solution that induced seizures was administered with the Hamilton syringe over 60 s.

Group 2 (Group R 1/5) (n=6): Using the same method as that used for Group C, 1/5 of the seizure dose of rocuronium

was added to the Ringer's lactate solution to obtain a total solution volume of 10 μL and administered to the rats.

Group 3 (Group R 1/10) (n=6): Using the same method as that used for Group C, 1/10 of the seizure dose of rocuronium was added to the Ringer's lactate solution to obtain a total solution volume of 10 μL and administered to the rats.

Group 4 (Group R 1/100) (n=6): Using the same method as that used for Group C, 1/100 of the seizure dose of rocuronium was added to the Ringer's lactate solution to obtain a total solution volume of 10 μL and administered to the rats.

Assessment of the effects of intracerebroventricular rocuronium bromide: A five-point scale was used to assess the CNS effects of rocuronium bromide administered intracerebroventricularly.⁸

0 = no observable effects

1 = decreased locomotor activity and/or piloerection

2 = agitation or shivering

3 = entire body tremors, posturing, or splayed limbs

4 = tonic-clonic convulsions or seizures

Termination of the study

The animals that had seizures were immediately sacrificed with an intraperitoneal injection of 120 mg/kg⁻¹ sodium thiopental. The animals that did not have a seizure were observed for 6 h and sacrificed with 120 mg/kg⁻¹ intraperitoneal sodium thiopental at the end of the study.

All the animals were injected postmortem with 50 μL of methylene blue via the permanent cannula into the cerebral ventricle, and the brain was bisected along the longitudinal fissure to examine whether the dye was uniformly distributed within the ventricle.

Statistical analysis

The statistical analyses were performed using the SPSS for Windows v. 11.0 statistical package. The results are expressed as the mean \pm standard deviation. The Kruskal-Wallis test, followed by the Mann-Whitney *U* and Fisher's exact tests, was used for the between-group comparisons. The within-group comparisons were conducted using Friedman and Wilcoxon tests, and $p < 0.05$ was considered statistically significant.

Results

Determination of seizure threshold (pilot study)

The rats in the control group, which received a total of 50 μL of Ringer's lactate solution (pH = 5.2) in the lateral cerebral ventricles, did not exhibit any behavioral alterations.

In the rocuronium group, the seizure threshold for ICV rocuronium bromide (pH = 3.6) was found to be $0.056 \pm 0.009 \mu\text{mol}$. The volume necessary to induce a seizure was found to be $35.0 \pm 5.48 \mu\text{L}$. The seizure threshold, as a function of the body weight of rats, was calculated as $0.286 \mu\text{mol}/\text{kg}^{-1}$ (Table 1).

Table 1 Rocuronium bromide doses and volumes.

Weight (g)	Dose (μmol)	Dose ($\mu\text{mol}/\text{kg}$)	Volume (μL)
201	0.064	0.326	40
198	0.064	0.331	40
212	0.064	0.308	40
206	0.048	0.239	30
198	0.048	0.248	30
184	0.048	0.267	30

The seizure threshold, as a function of the body weight of rats, was calculated as $0.286 \mu\text{mol}/\text{kg}$.

Table 2 CNS effects of rocuronium at 1/5 of the seizure threshold dose.

Weight (g)	CNS effect	Score
189	Seizure	4
266	Extremity gets a posture	3
232	Extremity gets a posture	3
238	Extremity gets a posture	3
199	Extremity gets a posture	3
184	Seizure	4

Table 3 CNS effects of rocuronium at 1/10 of the seizure threshold dose.

Weight (g)	CNS effect	Score
194	Shivering	2
180	Extremity posture	2
182	Extremity posture	2
217	Agitation	3
187	Agitation	3
189	Shivering	2

Evaluation of the dose-response results study (experimental study)

The pH values of the Group C, Group R 1/5, Group R 1/10, and Group R1/100 were determined to be 3.6, 3.9, 4.1, and 4.8, respectively.

In this study, we found that 1/5 of the seizure threshold dose typically caused splayed limbs, posturing, and entire body tremors, whereas 1/10 of the seizure threshold dose caused agitation and shivering (Tables 2 and 3). One-hundredth of the seizure threshold dose was associated with decreased locomotor activity (Table 4). The effects observed

Table 4 CNS effects of rocuronium at 1/100 of the seizure threshold dose.

Weight (g)	CNS effect	Score
218	Decreased locomotor activity	2
196	Decreased locomotor activity	1
192	Decreased locomotor activity	3
202	Decreased locomotor activity	1
186	Shivering	2
191	Decreased locomotor activity	1

at these doses gradually improved over a 1-h period and were then maintained as reduced locomotor activity. The animals were observed for a total of 6 h, during the time when feeding and motor behaviors became comparable to those of the control group.

Discussion

In this study, we found that rocuronium administered into the central nervous system of rats through the cerebral ventricles caused seizures when the rats were not under anesthesia and that the seizure threshold dose of rocuronium was $0.286 \mu\text{mol}/\text{kg}^{-1}$. We determined during the dose-response study that 1/5 and 1/10 of the seizure threshold dose of rocuronium yielded excitatory responses, while 1/100 of the dose resulted in decreased locomotor activity.

In the pilot study, behavioral changes were not observed in the rats when we administered $100 \mu\text{L}$ of Ringer's lactate solution at a rate of $5 \mu\text{L}/\text{min}^{-1}$. We administered a maximum of $40 \mu\text{L}$ while determining the epileptic seizure threshold dose. We do not hypothesize that the volume of the drug is responsible for the CNS changes observed in rats. In this study, we determined that the pH of the drug administered via the ICV cannula was not a contributing factor to the induction of seizures because only reduced locomotor activity was observed in Group 1.

In a similar study, Szenohradszky and colleagues administered atracurium, pancuronium, and vecuronium into the CNS and observed the side effects, finding the epileptic seizure potencies of atracurium > pancuronium > vecuronium (0.12 , 0.26 , and $0.46 \mu\text{mol}/\text{kg}^{-1}$, respectively).⁸ In the present study, the potency of rocuronium necessary to cause seizures was found to be near that of pancuronium.

In cases in which neuromuscular blocking drugs have been administered accidentally into the CSF,¹ the diffusion of rocuronium into the neuronal tissue from cerebrospinal fluid is likely a function of molecular weight, lipid solubility, and time.⁹ Based on the physical properties of rocuronium, it is likely to remain in the cerebrospinal fluid after injection, with rapid penetration into neuronal tissues being unlikely.⁹ Drugs in the cerebrospinal fluid can be redistributed into the peripheral circulation by transport across the blood-brain barrier. An ICV infusion may, in some instances, mimic a slow intravenous infusion.¹⁰ We did not observe such an incidence at any stage of the study, which may have occurred because the dose of rocuronium that entered the systemic circulation was not sufficient to reach the concentration necessary to reach the neuromuscular junction and cause muscle relaxation.

ICV-administered drugs may exert receptor-mediated effects at or near the brain-cerebrospinal fluid interface. Rocuronium is a quaternary ammonium compound. The positive charge of this molecule mimics that of the quaternary nitrogen atom of Acetylcholine (ACh). This atom is the major contributor to the effects of rocuronium on the neuronal nAChR.¹¹ There are insufficient data on the kinetics of the distribution of muscle relaxants in the CSF when administered into the cerebral ventricles,^{8,12} and it is not known in which part of the brain the ACh receptors

mediate the inhibitory and excitatory effects observed with rocuronium.

As with all muscle relaxants, the site of action of rocuronium is the postsynaptic ACh receptor at the neuromuscular junction. In an experimental study, Jonsson et al.¹³ demonstrated that rocuronium inhibited muscular and neuronal nAChR. Neuronal nAChR is irreversibly inhibited by rocuronium at the micro-molar level in a concentration-dependent manner. These findings suggest that rocuronium exerts only inhibitory effects on the ACh receptors in the neuromuscular junction, while it exerts inhibitory and excitatory effects on the nicotinic ACh receptors in the CNS.

Fuchs-Buder et al.⁶ and Tassonyi et al.¹⁴ reported that neuromuscular blocking agents may affect the CNS, as evidenced by the induction of apnea.

Shao et al.¹⁵ demonstrated that the cholinergic nervous system is important in the regulation of respiratory patterns, and inhibition of nACh may cause central depression of respiration. In our study, we observed no sign of depression of respiration or apnea.

The behavioral changes in rats manifested as decreased locomotor activity after 1 h and recovery of normal movement and feeding behavior after 6 h were attributed to rapid CSF clearance, which is $2.83 \mu\text{L}/\text{min}^{-1}$ in rats.¹⁶ Because the CSF volume in rats is $500 \mu\text{L}$, the total CSF clearance time is 176 min.^{4,8} Meulemans et al.¹⁷ reported that 45% of the CSF of a 290 g rat was cleared in 1 h, which is in accordance with our findings.

The exact mechanisms of action of muscle relaxants on the central nervous system have not been established. This study showed that rocuronium is effective on the CNS and has dose-related deleterious effects. Based on the results of this study, it is not possible to establish a relationship between the effects of rocuronium in humans and rats. The amount of rocuronium accumulation in the cerebrospinal fluid in patients receiving long-term rocuronium infusion is unknown.

It should be considered that CNS effects can be observed if rocuronium is infused over longer periods of time and in cases in which the blood–brain barrier is compromised or rocuronium is administered inadvertently during a regional block.

Conflicts of interest

The authors declare no conflicts of interest.

References

1. Peduto V, Gungui P, Di Martino M, et al. Accidental subarachnoid injection of pancuronium. *Anesth Analg.* 1989;69:516.
2. Sparr H, Wierda J, Proost J, et al. Pharmacodynamics and pharmacokinetics of rocuronium in intensive care patients. *BJA.* 1997;78:267–73.
3. Tobias J. Continuous infusion of rocuronium in a paediatric intensive care unit. *Can J Anaesth.* 1996;43:353–7.
4. Tassonyi E, Fathi M, Hughes GJ, et al. Cerebrospinal fluid concentrations of atracurium, laudanosine and vecuronium following clinical subarachnoid hemorrhage. *Acta Anaesthesiol Scand.* 2002;46:1236–41.
5. Savarese JJ, Caldwell JE, Lien CA, et al. In: Miller RD, editor. *Pharmacology of muscle relaxants and their antagonists, anesthesia.* 5th ed. Philadelphia: Churchill Livingstone; 2000. p. 412–90.
6. Cardone C, Szenohradszky J, Yost S, et al. Activation of brain acetylcholine receptors by neuromuscular blocking drugs: a possible mechanism of neurotoxicity. *Anesthesiology.* 1994;80:1155–9.
7. Fuchs-Buder T, Strowitzki M, Rentsch K, et al. Concentration of rocuronium in cerebrospinal fluid of patients undergoing cerebral aneurysm clipping. *BJA.* 2004;92:419–21.
8. Matteo RS, Pua EK, Khambatta HJ, et al. Cerebrospinal fluid levels of d-tubocurarine in man. *Anesthesiology.* 1977;46:396.
9. Segredo V, Matthay MA, Sharma ML, et al. Prolonged neuromuscular blockade after long-term administration of vecuronium in two critically ill patients. *Anesthesiology.* 1990;72:566–70.
10. Hennis PJ, Fahey MR, Canfell PC, et al. Pharmacology of laudanosine in dogs. *Anesthesiology.* 1986;65:56–60.
11. Szenohradszky J, Trevor AJ, Bickler P, et al. Central nervous system effects of intrathecal muscle relaxants in awake rats. *Anesth Analg.* 1993;76:1304–9.
12. Vassilikos D, Tsakiliotis S, Veroniki F, et al. Inadvertent epidural administration of cisatracurium. *EJA.* 2004;21:663–72.
13. Kostopanagiotou G, Mylona M, Massoura L, et al. Accidental epidural injection of vecuronium. *Anesth Analg.* 2000;91:1550–1.
14. Cesur M, Alici H, Erdem A, et al. Accidental caudal injection of rocuronium in an awake patient. *Anesthesiology.* 2005;103:444–5.
15. Stoelting RK, Miller RD. *Neuromuscular blocking drugs, anesthesia.* 4th ed. Philadelphia: Elsevier Health Sciences; 2000. p. 89–106.
16. Bartkowski RR, Witkowski TA, Azad S, et al. Rocuronium onset of action: a comparison with atracurium and vecuronium. *Anesth Analg.* 1993;77:574–8.
17. Khalil M, D'Honneur G, Duvalet P, et al. Pharmacokinetics and pharmacodynamics of rocuronium in patients with cirrhosis. *Anesthesiology.* 1994;6:241–5.