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Effect of the ketamine/xylazine anesthetic on the auditory brainstem response of adult gerbils

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Abstract

The auditory brainstem response (ABR) is a test widely used to assess the integrity of the brain stem. Although it is considered to be an auditory-evoked potential that is influenced by the physical characteristics of the stimulus, such as rate, polarity and type of stimulus, it may also be influenced by the change in several parameters. The use of anesthetics may adversely influence the value of the ABR wave latency. One of the anesthetics used for ABR assessment, especially in animal research, is the ketamine/xylazine combination. Our objective was to determine the influence of the ketamine/xylazine anesthetic on the ABR latency values in adult gerbils. The ABRs of 12 adult gerbils injected with the anesthetic were collected on three consecutive days, or a total of six collections, namely: pre-collection and A, B, C, D, and E collections. Before each collection the gerbil was injected with a dose of ketamine (100 mg/kg)/xylazine (4 mg/kg). For the capture of the ABR, 2000 click stimuli were used with rarefaction polarity and 13 stimuli per second, 80 dBnHL intensity and in-ear phones. A statistically significant difference was observed in the latency of the V wave in the ABR of gerbils in the C and D collections compared to the pre-, A and E collections, and no difference was observed between the pre-, A, B, and E collections. We conclude that the use of ketamine/xylazine increases the latency of the V wave of the ABR after several doses injected into adult gerbils; thus, clinicians should consider the use of this substance in the assessment of ABR.

Key words: Auditory-evoked potential; Auditory brainstem response; Anesthetics; Ketamine; Xylazine

Introduction

The brain stem auditory-evoked potential or auditory brainstem response (ABR) is a test used in clinical practice. The patient must be still in order to avoid the creation of electrical artifacts that would interfere with the detection of the waves and their interpretation. Some patients are difficult to evaluate under these conditions and often need to be sedated (1). Although this potential is considered to be influenced by the physical characteristics of the stimulus, such as rate, polarity and type of stimulus, the use of anesthetics can also alter the synaptic transmission, and this may be considered an adverse condition for testing the ABR (2).

Anesthesia acts by depressing the central nervous system, and there are several types of anesthetics, each one with specific pharmacodynamics. One type of anesthetic is ketamine, used mainly in animal research. Ketamine acts as a non-competitive antagonist of N-methyl-D-aspartate (NMDA), one of the glutamate receptors. It interferes with the action of NMDA, blocking the open channel and/or binding to the closed channel, thus reducing the opening frequency of the channel. A consequence is a reduction of the action of glutamate, which is essential for neuronal survival (3). Ketamine can also be combined with xylazine, a painkiller and muscle relaxant. Xylazine is an agonist of alpha 2 adrenergic receptors located presynaptically. These receptors reduce noradrenaline release by inhibiting the influx of calcium in the neuron, with a consequent hypotensive and tranquilizing effect (4).

Literature reports of ABR and the use of ketamine (Ketalar) combined or not with xylazine (Rompun) are controversial. Some investigators report no influence of this anesthetic on the V wave latency values (5-7), while

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others report a change in ABR wave latency with the use of ketamine, with an increase of wave latencies and amplitude change (2,8,9).

Therefore, the objective of the present study was to investigate the influence of the ketamine anesthetic combined with xylazine in adult gerbils by measuring the latency of the ABR's V wave at several fixed times after injection of the anesthetic.

Material and Methods

The study was approved by the Ethics Committee for the Analysis of Research Projects, Clinical Council, Hospital das Clínicas, Faculdade de Medicina, Universidade de São Paulo (protocol No. 0456/09). The research was conducted in the Laboratory of Medical Investigation of Clinical Emergencies, Faculty of Medicine, University of São Paulo.

The study population consisted of 12 adult male gerbils from the animal facilities of the Clinical Department, Faculty of Medicine, University of São Paulo, weighing 56 to 79 g (average weight 69 g), aged 3 to 5 months, maintained under controlled environmental conditions on a 12/12-h light/dark cycle, temperature of 22°-27°C, 45 to 65% humidity and a sanitized environment, with free access to common feed and water.

An auditory-evoked potential system (Navigator model, two-channel, Bio-Logic System Corp., USA) was used for the electrophysiological assessment. The stimuli were presented through 3B in-ear phones positioned in the left ear of the animals, while the right ear was unobstructed.

The ABR was recorded with three electrodes positioned on Cz (according to the international 10-20 system) and left mastoid M1 (10) and a common electrode. The left mastoid was used as a reference, and the animal's foot was used as the common electrode.

Before placing the electrodes, the hair was removed and an electrolytic paste was applied to the animals' skin to provide better impedance. After the electrodes were placed, the impedance was checked and was maintained at a level below 5 KΩ.

For the capture of the ABR, 2000 stimuli of the click type were used with rarefaction polarity and at a rate of 13 stimuli per second, 80 decibels hearing level (dBnHL) intensity and in-ear phones. The responses were captured, amplified, scanned, averaged out, and filtered using a 100- Hz low-pass filter and a 1500-Hz high-pass filter. The registration window used was 10.66 ms after the stimulation. All responses were reproduced, therefore ensuring the reproducibility of the potential acquired.

The range of audibility for the gerbils used lies between 1 and 20 kHz (11); therefore, the click proved to be effective for the assessment of hearing in these animals.

Prior to data recording, the animals were anesthetized with a peritoneal injection of 100 mg/kg ketamine (Parke-Davis, Brazil) and 4 mg/kg Rompun (active principle: xylazine; Bayer HealthCare, Brazil). This protocol has already been used by a group of the Laboratório de Emergências Clínicas da Faculdade de Medicina.

First, electrophysiological assessment (ABR) was performed prior to any procedure (pre-collection, performed on day 1), so that these measures could serve as the baseline for all other measurements performed. Next, the ABR collections were performed at the following times: day 2, first collection, collection A; second collection, collection B, 2 h after collection A; third collection, collection C, 4 h after collection A, and fourth collection, collection D, 8 h after collection A; day 3, collection E, 24 h after collection A. The anesthetic was injected prior to all collections. After the last collection, all animals were sacrificed according to the protocol of the Ethics Committee of the institution. For ABR assessment, only the V wave was considered.

For this study, we compared the values of the ABR's V wave in the same animal - intra-group analysis - at the different collection times. Data were analyzed statistically by the Friedman test (12), with the level of significance set at 5% ($P < 0.05$).

Results

Table 1 shows V wave latencies of the ABR at all time points studied. There was a difference in the baseline (pre) and A and E collection values compared to the B, C, and D collections.

Figure 1 shows the average latency value for the V wave of the ABR, obtained by the "grand-averaged" method in the study group at all times of collection (pre-collection, A, B, C, D, and E collections). Table 2 shows the P value indicating significance or non-significance between the ABR values at the different times of collection.

This analysis shows the effect of the anesthetic on the

Table 1. V wave latencies of the auditory brainstem response in the study group at all times of collection.

Collection	Latency
Pre	3.03 ± 0.18
A	3.00 ± 0.13
B	3.45 ± 0.50
C	3.95 ± 0.61
D	3.63 ± 0.55
E	2.98 ± 0.23

Data are reported as average ± SD for 12 gerbils. Pre = day 1 (baseline); A = day 2 (first collection); B = 2 h after collection A (second collection); C = 4 h after collection A (third collection); D = 8 h after collection A (fourth collection); E = day 3, 24 h after collection A (fifth collection).

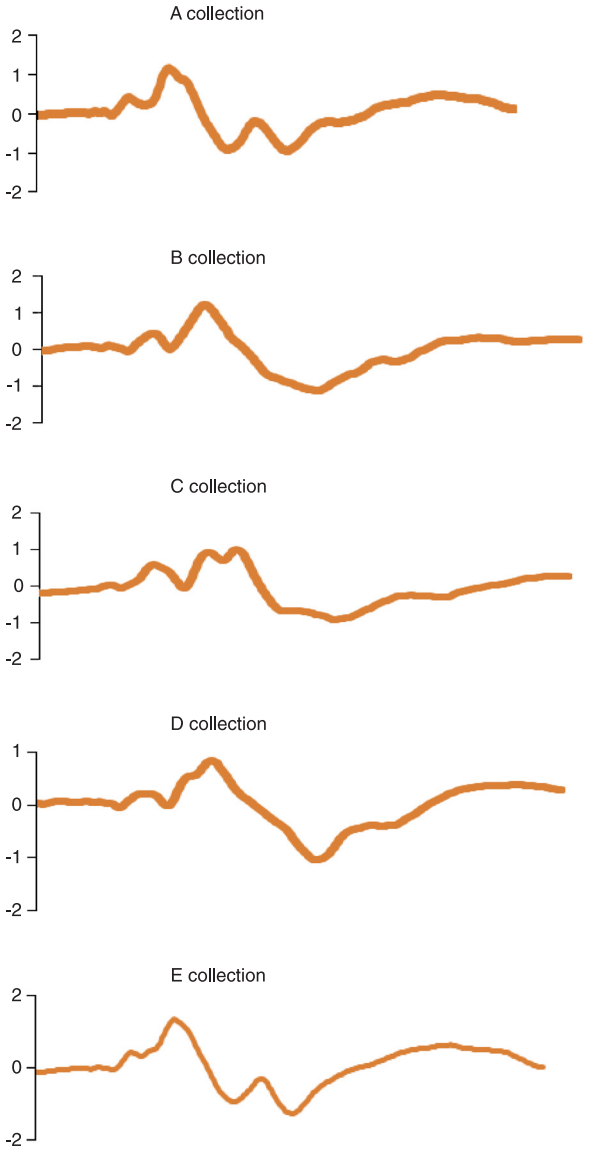

Figure 1. Examples of auditory brainstem response recordings of the study group at the different times of collection. See legend to Table 1 for explanation of groups.

group. We observed a significant difference ($P \leq 0.05$) in V wave between points pre and C, pre and D, A and C, A and D, C and E, and D and E in the group. These collections were performed on the second day, when four doses of anesthetic were used, which means that there was an accumulation of ketamine in the animal.

Discussion

In clinical practice, anesthetics are used for the ABR procedure at surgical centers and hospitals. Patients who are difficult to evaluate need to be sedated for the procedure

Table 2. Intra-group comparison of the average latencies of the V wave of the auditory brainstem response at the different times of collection.

	Pre	A	B	C	D
Pre	-	-	-	-	-
A	NS	-	-	-	-
B	NS	NS	-	-	-
C	0.000*	0.000*	NS	-	-
D	0.028*	0.007*	NS	NS	-
E	NS	NS	NS	0.000*	0.005*

NS = non-significant. See legend to Table 1 for explanation of groups. *P < 0.05 (Friedman test).

(1). In addition, this potential is used for surgical monitoring (13), when the patient is anesthetized and often presents hypothermia (14) and also in situations in which the patient is evaluated after the surgical procedure, still under the effect of anesthesia.

In this study, we used the ketamine-xylazine anesthetic to assess the value of the V wave in ABR. According to the data in Table 1, there was an increase of the ABR's V wave between the collections in which there was an accumulation of anesthetic in the animal, that is, collections C and D. The C collection was the third collection recorded on the second day, after two injections of anesthetics given 2 and 4 h earlier. The D collection was the fourth collection on the second day, with an interval of 4 h after the last injection of the anesthetic. This explains the decrease in latency value for the V wave of ABR in the C collection, but there was still the cumulative presence of drugs injected into the body of the animal. A statistically significant difference can be seen in data from Table 2. It is also possible to observe that on the third day of collection (collection E) the average latency value of the V wave in the group was very close to that of the initial collection (pre-collection), confirming even further the influence of the drug injected, as the latency values return to baseline.

This finding supports other studies that used the same combination of anesthetics and found increased responses in the ABR of cats and mice (2,8,9). These previous studies, despite using different anesthetic concentrations, are compatible with the results obtained in the present study.

This extension of wave in the ABR following the use of the ketamine anesthetic can be explained by the effect of the drug in blocking the NMDA receptor channel. When this occurs, there is a reduction in the glutamate action and, consequently, a reduction in synaptic transmission (3). Regarding the xylazine anesthetic, some studies with other alpha 2 adrenergic receptors, such as clonidine (15) and dexmedetomidine (16-18), have demonstrated a minimal effect of these drugs on the response-evoked potentials. This suggests that in the present study the results were

mainly due to the action of ketamine.

Thus, the results of this analysis show the effect of the use of ketamine-xylazine anesthetics on the latency of the V wave in ABR, revealing an increased response. It is important to note that this type of anesthetic is used in animal and, therefore, other studies involving anesthetics used in humans need to be conducted. It is also important to consider the amount of drug injected, that is, successive doses within a short time or large doses for events requiring longer sedation, as in the case of prolonged surgical procedures. Therefore, clinicians should consider the use of ketamine in the assessment of ABR.

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