Effect of Two Doses of Dexmedetomidine on Index of Consciousness and Permutation Entropy in Rabbits

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Abstract. The Index of Consciousness (IoC) and the Permutation Entropy (PE) are the most recent EEG-based indexes for depth of anesthesia monitoring but their use during ketamine or dexmedetomidine anesthesia has never been reported. In this study, the ability of these measures o differentiate between the effects of two different doses of dexmedetomidine combined with a fixed dose of ketamine in rabbits was studied.
Five adult female rabbits received one of two ketamine/dexmedetomidine combinations (15/0.025 and 15/0.05 mg/kg: G0.025 and G0.05, respectively). The effect of the two doses on IoC and PE were compared.
The effect of dexmedetomidine dosage on the EEG was not significantly different between groups for any of the studied indexes (IoC ($p=0.058$); PE ($p=0.392$)). Only the IoC showed a significantly lower value in G0.05 than G0.025 at T15. The analysis revealed a significant effect of time on the IoC ($p=0.001$) and PE ($p=0.0195$).
IoC and PE did not decrease to values correspondent to the anesthetized state, as was expected regarding previous works. This may be explained by the known excitatory effects of ketamine on the EEG. However, the tendency of the indexes to decrease after induction and to be lower in the animals that received the higher dexmedetomidine dosage suggests the capacity of these parameters to detect depressant effects of alpha-2-agonists.

1 Introduction

Ketamine is widely used in veterinary practice for anesthesia, sedation, and analgesia. The cardiovascular and respiratory stability associated with its administration, the fact that it is well absorbed by different administration routes and its adequacy to be used in different animal species, make it a very safe and practical drug [3-4]. It causes a state of dissociation, rather than unconsciousness or hypnosis, by blocking the activation of non-competitive N-methyl-D-aspartate (NMDA) receptors [5]. This state is characterized by sensory loss, analgesia and amnesia. However, muscular tonus persists which may be a limitation during surgery. Ketamine is often associated with alpha-2-agonists to produce general anesthesia in animals [6]. In the past, xylazine and medetomidine were the most used alfa-2-agonist drugs. Medetomidine is a potent, selective, and specific alpha 2-adrenoceptor agonist that compensates for the poor muscle relaxant and analgesic effects of ketamine, while the cardiac stimulating pro-

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perties of ketamine partially compensate the medetomidine-induced bradycardia [6]. More recently, dexmedetomidine was introduced in human and veterinary anesthesia. It is an isomer of medetomidine that when administered at half the dose, induces similar effects as medetomidine [7]; [8].

EEG-derived indexes have become popular as a method to assess DoA in human patients. These indexes are derived from the EEG by mathematical algorithms and some are available in the form of commercial monitors [9]. The bispectral index (BIS) is the most widely known of these monitors. Several studies in animals tried to assess the potential of this monitor to reflect DoA in animals, however, results are generally contradictory and the fact that the BIS calculation incorporates an extensive database of EEG's from anesthetized humans may limit its application in animals [10]. More recently, methods based in the analysis of non-linear time series were introduced. The Index of Consciousness (IoC) is a commercial index based in symbolic dynamics for the derivation of a number from the EEG that correlates with the DoA of humans [11]. By the other side, Permutation Entropy (PE) is an open-source parameter which showed a high resistance to artifacts and a good capacity to reflect DoA during sevoflurane and propofol anesthesia in humans [11] and had better performance than other parameters during isoflurane and propofol anesthesia in rabbits [1]; [2] and rats [12]. No reports on the use of these indexes during ketamine administration are available, neither in humans or animals. It has been shown that ketamine produces excitation of the EEG, and has been associated with a pronounced increase in DoA indexes, such as the BIS and parameters derived from the power spectrum [10]. By the other hand, alpha-2-agonists produce depression of the cortical activity causing decreases in EEG-derived indexes [10].

In this work, the capacity of IoC and PE to detect different doses of dexmedetomidine combined with a fixed dose of ketamine was evaluated in rabbits.

2 Methods

Five adult female New Zealand White rabbits weighting 4.2+ 0.85 kg received one of two ketamine/dexmedetomidine combinations (15/0.025 and 15/0.05 mg/kg: G0.025 and G0.05, respectively) given by intramuscular injection on two successive occasions with a 15 day interval.

2.1 Brain Waves Recordings

Extracranial EEG was recorded with electrodes placed non-invasively on the rabbits head skin using the IoC-View monitor (Aircraft Medical (Barcelona), Barcelona, Spain) (Fig. 1).

The IoC-View monitor electrodes were placed directly on the head skin after careful preparation. In the fully awake animals, the heads were shaved, cleaned, and surface layers removed with fine sandpaper and acetone. The electrodes used were pregelled single-use silver/silverchloride electrodes with liquid gel and the surrounding adhesive made of medical-grade acrylate. The electrode DC-offset was typ. 1 mV and AC-impedance typ. 150 Ohm (Swaromed, Innsbruck, Austria). Three of these elec-

trodes were applied to record the EEG. Two electrodes were placed 1 cm caudal to the lateral eye cantus (one for each eye); a central electrode was placed on the midline on the frontal bone 3 cm away from each previously applied electrode (Fig. 2).

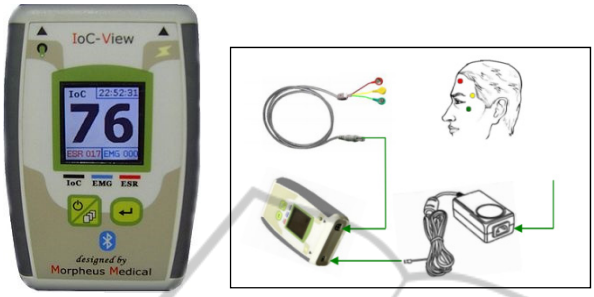

Fig. 1. IoC-View monitor (Aircraft Medical (Barcelona), Barcelona, Spain), shown on the left. The techniques for connection of electrodes to the monitor and placement of the electrodes on the forehead of a human patient are shown on the right [13].

This localization was based in previous works in rabbits [1]; [2] and has been concluded to give the best quality EEG signal after testing different positions in pilot studies with the IoC-View monitor.

Impedance was automatically checked by the monitor and maintained below 15000 Ohms at 1024 Hz. The electrodes were connected to the IoC-View monitor, which was connected by Bluetooth to a personal computer with the IoC-View graph software version 1.4 installed, a storage software provided by the manufacturer.

2.2 Anesthetic Monitoring

After EEG baseline recording in the fully awake animals during 5 minutes, the fur on the ears was clipped and the skin cleaned with alcohol and a local analgesic cream was applied to the ears skin (EMLA, Nycomed US Inc, New York, USA). Thirty minutes after, two 22G catheters were placed, one in the marginal ear vein and another into the central ear artery for arterial pressure monitoring. Both auricular catheter systems were flushed with heparinized saline and fixed to the skin. The animals were then oxygenated with a facial mask at 5L/min for 5 min.

During anesthesia, the rabbits were placed in ventral recumbence above a heating blanket and rectal temperature was continuously monitored and maintained between 37 and 38ºC.

Anesthetic monitoring included cardio respiratory monitoring provided by a Datex S/5 Anesthetic station (Datex Ohmeda, Helsinki, Finland) which included: pulse-oxymetry and pulse rate monitored with the probe placed in the ear, invasive mean arterial pressure (MAP), inspired and end-tidal concentrations of oxygen and carbon dioxide.

Clinical monitoring was also performed and included: nociceptive reflexes and respiratory rate monitoring. Respiratory rate was assessed visually, by counting the number of respiratory movements per minute. The ear pinch reflex was the nociceptive reflex evaluated.

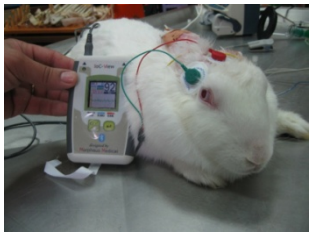

Fig. 2. Gel-coated silver-silver chloride electrodes (Swaromed, Innsbruck, Austria) applied on the rabbits head during recording of the electroencephalogram with the IoC-View monitor.

At the end of the anesthesia procedure fresh gas flow rate was increased to 5L/min of 100% oxygen until the rabbits regained swallowing reflexes and at this point extubation was performed. Animals were considered recovered from anesthesia when they exhibited an alert stance and had regained ambulation and limbs coordination.

2.3 Signal Processing

2.3.1 Digitization and Filtering

EEG data were recorded by the IoC-View monitor as binary files and were exported to Matlab using code provided by the manufacturer.

Before the analysis of the EEG, careful visual inspection was performed to select only epochs that were free from visually detected artifacts.

The original sampling frequency of the EEG was 1024 Hz. It was digitized to 256 Hz before processing using the Matlab function "*downsample.m*". This allowed a faster computation of the indexes of DoA. Removal of the mean value of the signal in order to get out any threshold was performed in all signals recorded.

A Butterworth of 8^{th} order digital filter was used with cut off frequencies between 0.5 and 32 Hz before the derivation of PE.

2.3.2 Calculation of Permutation Entropy (PE)

PE was first described by Bamdt and Pompe [14] as a method for ordinal pattern analysis of non-linear time series. It analyzes consecutive subvectors of constant length (m) in the analyzed signal interval (length N). Then, it orders the samples in every subvector according to their amplitude and defines permutations of order m (m!). The parameter value is given by the resultant normalized probability distribution of the obtained permutations, using the Shannon entropy formula. In this study, we used m=3 and N=2048.

In more detail it is calculated in the following steps:

1- Fragments the continuous EEG signal into a sequence of motifs with a determined length (examples shown in grey).

2- Identifies each motif as belonging to one of the six possible types according to their shape Counts the number of motifs from the real EEG that belongs to each of the six categories, to obtain the probability of occurrence of each motif in the signal (p_i)

3- Calculates the PE of the resultant normalized probability distribution of the motifs, using the standard Shannon uncertainty formula (1):

$$\mathrm{PE} = \frac{-\sum_{j=1}^{J} pj \ln pj}{\ln(number\ of\ motifs)} \quad (1)$$

2.3.3 Index of Consciousness (IoC)

The IoC-View monitor was used for EEG recording and directly derived the IoC as well as the electromyographic activity (EMG), the electroencephalogram suppression ratio (ESR) and the signal quality index (SQI). The calculation of the IoC is not totally understood. It is based in the symbolic dynamics method which transforms a time series into a symbol sequence in order to reveal the non-linear characteristics of the EEG. It also integrates the beta-ratio (frequency range between 11 and 42 Hz) during superficial anesthesia and the ESR. These components are then combined using a proprietary discriminatory function to produce the final index IoC. As previously described, in humans, decreasing values of IoC correspond to gradually loss of consciousness and a deepening of the level of anesthesia. In a unitless scale from 99 to 0, an index of 99 indicates an awake patient and an index of 0 indicates a flat EEG.

The monitor includes an EMG filter that eliminates most of the potential interfering electromyographic activity before the derivation of the IoC and calculates the EMG which is given as a percentage and shows the energy of the EMG level in the 30–45 Hz frequency band [11].

2.4 Statistical Analysis

The parameters were studied in the following times: baseline recordings in the awake animals (T0), and at intervals of 5 minutes after administration of anesthesia (from T5 to T35). Statistical analysis consisted in two-way ANOVA with Bonferroni correction for multiple comparisons to check for differences between the two groups in IoC, PE, EMG, mean arterial pressure (MAP – mmHg), heart rate (HR - beats per minute - bpm), respiratory rate (RR – respirations per minute - rpm) and body temperature (T - ºC) and for changes throughout study periods. P-value <0.05 was considered significant.

3 Results

Loss of ear pinch reflex happened in all animals of G0.05 and only in one rabbit in G0.025. The typical raw EEG recordings during the first twenty minutes are sown in Fig.3. The correspondent power spectrum analysis for each EEG fragment is shown in Fig.4. There were no clear differences in the raw EEG from T0, T5, T10 and T15. However, power spectral analysis revealed a decrease in frequency from T0 to T10, and an increase from T15 to T20 minutes (Fig.4). These results are represented by the spectral edge frequency 95%.

The behavior of the IoC, PE and EMG throughout study times is shown in Fig.5. The effect of dexmedetomidine dosage was not significant for any of the studied in

dexes (IoC (p=0.058); PE (p=0.392)) neither for the EMG (p=0.056). Only the IoC showed a significantly lower value in G0.05 than G0.025 at T15. The analysis revealed a significant effect of time on the IoC (p=0.001), PE (p=0.0195) and on EMG (p<0.001).

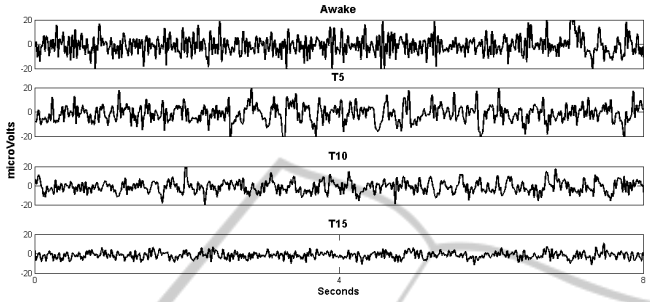

Fig. 3. Raw EEG changes in the group of animals that received the higher dose of dexmedetomidine (0.05 mg/kg) combined with a fixed dose of ketamine. EEG fragments recorded in the awake animals (Awake) and in the following 15 minutes after ketamine and dexmedetomidine administration in the higher dose group. Eight seconds EEG fragments are shown.

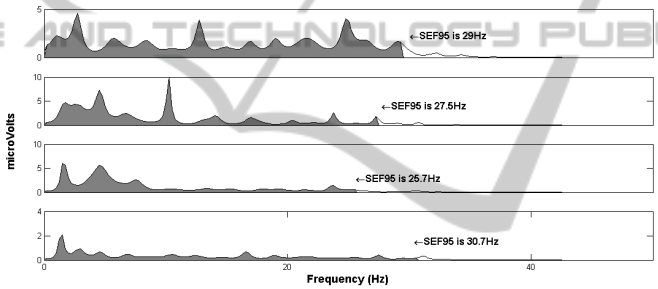

Fig. 4. Power spectrum analysis of the EEG fragments shown in figure 3 from the group of animals that received the higher dose of dexmedetomidine (0.05 mg/kg) combined with a fixed dose of ketamine. The spectral edge frequency 95% (SEF95) is shown for the awake animals (Awake) and in the following 15 minutes after ketamine and dexmedetomidine administration in the higher dose group.

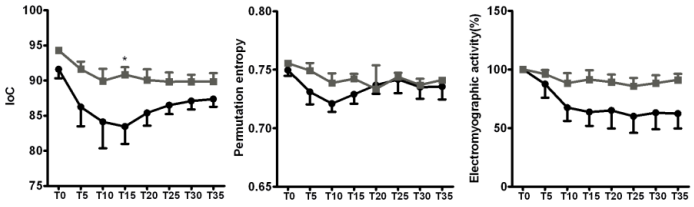

Fig. 5. Index of consciousness (IoC), electromyographic activity (%) and permutation entropy at the study times: baseline recordings in the awake animals (T0), and at intervals of 5 minutes after administration of anesthesia (from T5 to T35). The mean and standard deviation are presented (N=5). * existence of significant differences between the two groups (p<0.05). The black line represents the group of animals that received the higher dose of dexmedetomidine (0.05 mg/kg) and the grey line represents the group of animal that received the lower dose of dexmedetomidine (0.0025 mg/kg).

Regarding the cardiorespiratory parameters, the only differences found were in HR which was significantly lower in G0.05 from T10 to the end of the study and also RR that was significantly lower in the same group at T25 (Table 1).

Table 1. Clinical data: heart rate (HR –bpm), mean arterial pressure (MAP – mmHg), respiratory rate (RR – rpm) and body temperature (T - ºC) recorded at the study times: baseline recordings in the awake animals (T0), and at intervals of 5 minutes after administration of anesthesia (from T5 to T35). The mean and standard deviation are presented (N=5). G0.025 – group that received the lower dose of dexmedetomidine (0.025 mg/kg); G0.05 – group that received the higher dose of dexmedetomidine (0.05 mg/kg). * existence of significant differences between the two groups ($p<0.05$).

Variable	Group	T0	T5	T10	T15	T20	T25	T30	T35
HR (bpm)	G0.025	242±15,1	234±17	214±22,3	210±19,3	211±15,5	200±18,2	199±14,3	197±14,5
	G0.005	240±9,9	215±34,1	200±20,8*	187±16,5*	182±11,2*	182±9,9*	176±8,7*	176±12,4*
MAP (mmHg)	G0.025	89±5,6	81±11,9	71±10,6	63±11,7	63±9,2	58±10,1	57±10,9	55±9,2
	G0.005	103±7,7	83±5,8	72±8,1	69±11,2	67±10,9	65±10,1	64±9,1	62±9,9
RR (rpm)	G0.025	178±43,8	100±56,4	104±53,9	99±34,2	90±35,2	88±25,1	98±33,2	97±26,9
	G0.005	186±26,2	114±41,4	91±15,3	79±13,4	84±15,2	81±7,2*	86±8,3	87±15,1
T (ºC)	G0.025	38,6±1,03	38,5±0,78	38,2±1,21	38,2±0,99	38,2±0,79	38,1±0,84	38,1±0,83	38,3±0,83
	G0.005	39,0±0,10	39,1±0,22	39,0±0,22	38,9±0,30	39,0±0,17	38,8±0,24	38,8±0,22	38,7±0,29

4 Conclusions

In this study, the capacity of the Index of Consciousness (IoC) and the Permutation Entropy (PE) to reflect the EEG effects of two doses of dexmedetomidine combined with a fixed dose of ketamine was evaluated. No significant differences between the two doses of dexmedetomidine were found regarding IoC and PE, although a tendency could be suggested by the p-value obtained for the IoC of 0.058.

Clinically, there were clear differences between the two groups of animals: the ear pinch reflex was lost in all the animals that received the higher dose of dexmedetomidine, heart rate was significantly lower in this group since ten minutes after induction of anesthesia, and EMG had a tendency to be lower in the same group, reflecting higher muscle relaxation in animals that received higher dose of dexmedetomidine. Ketamine was administered in the same dose in both groups. This drug has been shown to cause increases in the frequency components of the EEG and in the value of EEG-derived indexes [9]. The presence of ketamine on the anesthetic protocol could have limit the capacity of IoC and PE to detect different dexmedetomidine depths of anesthesia. The present results showed that, although increases in dexmedetomidine dose produced deeper anesthetic levels, this was not reflected in the values of EEG-derived indexes PE and IoC. However, the IoC showed a tendency to differ between the two groups. EEG-derived indexes in animals should thus be adapted to the protocol used and caution in the interpretation of these non-linear times series parameters during ketamine administration should remain, as when using frequency domain parameters. The rabbit as used in this study seems to be a promising animal model for the study of drug effects on the non-invasively recorded EEG, as it allows the recording of a good quality signal.

Acknowledgements

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