RESPIRATORY RATE MEASUREMENTS FROM TELEMETRY BLOOD PRESSURE IN MALE CYNOMOLGUS MONKEYS

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ABSTRACT

A blood pressure analysis program was evaluated for its ability to derive respiratory rate from a telemetry (Open-A.R.T./Ponemah: DSI/Gould) blood pressure waveform in cynomolgus macaques. The test utilized 2 male cynomolgus monkeys that had been surgically implanted with telemetry transmitters (DSI #TL11M2-D70-PCT) ten months earlier. The animals were sedated surgical prepared and maintained on isoflurane gas anesthesia. An arterial catheter was inserted through the contralateral femoral artery so that the pressure transducer catheter tip was approximately the same location in the dorsal aorta as the telemetry catheter. Each animal was then subjected to stimuli designed to increase or decrease respiratory rate. The stimuli included hypothermia, mechanical hyperventilation, Dopram V®, elevated body temperature and sodium pentobarbital.

Respiratory rate was evaluated at frequent intervals before and after various stimuli. A direct respiratory count was performed by a trained observer and compared to the respiratory rate measured by the telemetry system. The algorithm monitors the low rolling oscillations super imposed on the systolic blood pressure as the animal breaths in and out. The algorithm parameters of Minimum Pulse Height, Pressure Drop and Respiration Smoothing were empirically determined by monitoring these animals while free roaming in their cage.

The telemetry system accurately measured mechanical ventilation, hyperventilation, apnea, shallow respiration and transitory increase in respiratory rate following Dopram V® infusion. The overall correlation coefficient for this study was r=0.95. During apnea episodes following mechanical hyperventilation, heart rate and blood pressure remained constant but the respiration tracing of the telemetry system went flat and no breaths were counted by either the observer or the telemetry system. Thus we were able to demonstrate that the telemetry system could accurately monitor respiration at both high (50 Breath Per Minute (BPM)) and low respiratory rates (2 BPM) including apnea.

INTRODUCTION

Telemetry Systems allow us to measure sensitive biological parameters in animals that are conscious, unrestrained and maintained in an environment free from the stress of human presence. The parameters measured in this way are converted into electronic signals and sent to a receiver placed in the animal cage. The signal is then translated back into physiological parameters. The physiological signal can be displayed in real time on a screen and captured electronically for processing by a computerized analysis system.

Telemetric instrumentation in cynomolgus monkeys allows assessment of several vital systems contained within the Core Battery (e.g. Cardiovascular System, Respiratory System) as defined by the ICH guidelines S7A and S7B.

The system utilized to acquire and evaluate respiratory rate data in this study includes acquisition and analysis systems from Data Sciences International (DSI) and Gould Ponemah. The system also includes a Data Security Option (DSO) that handles data tracking and other security issues.

The purpose of this study was to evaluate the ability of the Telemetry System to consistently and accurately detect and display respiratory rate derived from blood pressure over the expected physiological and pathological ranges in an actual animal test system. To achieve this goal, respiratory rate was simultaneously measured by visual and derived methods in these same cynomolgus monkeys. Each test was held to strict standards for precision and accuracy established by SNBL USA Ltd.

MATERIALS AND METHODS

Two cynomolgus monkeys that had been implanted with TL11M2-D70-PCT (DSI) transmitters 10 months previously were utilized in this study. Before the study, each animal was fasted, sedated with ketamine plus xylazine, intubated, and maintained on isoflurane anesthesia. The animal was placed on a polycarbonate surgical table with a telemetry receiver placed on a smaller shelf beneath the table. A circulating water blanket was placed under the animal to help maintain body temperature. The animal was placed in a supine recumbent position with the head slightly elevated and the tail resting on the table. The animal was covered with standard surgical drapes, supplemental heat was supplied using an air warmer (BairHugger®). Hot water bottles placed next to the animal's abdomen and thorax to simulate an increase in body temperature. Supplemental heat and drapes were removed as necessary to achieve the desired reduction in body temperature.

Dopram V® (Dopram) was administered into an IV drip to stimulate respiratory rate and heart rate. Epinephrine (Epi) infusion (1 mL into 100 mL of 0.9% Sodium Chloride) was started at 20 mL/ hr and was increased to 50 mL/ hr to achieve a substantial increase above the resting heart rate.

After these manipulations, the Isoflurane vaporizer setting was increased ~0.1% every 3 to 7 minutes until it reached 4% or until the heart rate was depressed to approximately 50% of the normal rate.

The animal was then given an IV injection of Euthanasia solution diluted 1:4 with Saline. The IV injection was initially started at 0.1 mL and then repeated with an additional 0.1 mL every 3 to 5 minutes until the animal stopped breathing. The animal was euthanized with a pentobarbital overdose (~1.5 mL of Euthasol solution).

Direct respiratory counts were performed by a trained observer and compared to the respiratory rate measured by the telemetry system. The gas anesthesia machine had a re-breathing bag that was used when chest movements were not visible to monitor respiratory rate. The trained observer sat close to the animal with a stopwatch and recorded the number of breaths from either the chest or re-breathing bag movements over a 30 second interval every 3 to 5 minutes during the critical periods. The 30-second counts were converted to Breaths per Minute (Bpm). Blood pressure (BP) waveforms were recorded and analyzed for the respiratory pressure oscillations superimposed on the systolic blood pressure by the Ponemah Physiology Platform (P3) computerized analysis system. The telemetry algorithm monitors the oscillations superimposed on the systolic blood pressure as the animal breaths in and out. The algorithm parameters of Minimum Pulse Height, Pressure Drop and Respiration Smoothing were empirically determined by monitoring these animals while free roaming in their cage. The telemetry system was also set to accumulate breaths over each 30-second period (i.e. 30 second logging rate). The visual time interval data was then correlated with the telemetry interval by covariance analysis in Microsoft Excel® 2000 (9.0.2720).

To evaluate the respiratory rate in conscious free roaming animals, previously Implanted animals were acclimated to the telemetry habitat and daily routine. The animals were not on treatment and data was collected continuously for one week at a logging rate of one hour. The results were compared for normal variation.

The blood pressure waveforms were sampled at 1000 Hz. All telemetry recordings were continuous from the start of manipulations until after the end of manipulations.

RESULTS

Sedated Comparison: Initial resting respiratory rate was between 25 and 30 Bpm for both animals (Figure 1 and 2). Both animals showed chrematistic changes in respiratory rate based on the specific stimuli. The direct counts and the respiratory rate derived from blood pressure were strongly correlated throughout the full range of respiratory rates (Figures 1 and 2). The rates ranged from apnea to over 50 breaths per minute. The first animal (No 1) showed what appeared to be a compensatory increase in respiratory rate in response to the hot water bottles placed next to the animal's abdomen and thorax and that resulting increase in body temperature (Figure 1; temperature in the companion poster). The second animal did not receive the appropriate anesthesia initially and so respiratory data was not analyzed for 30 minutes (Gap in Figure 2). The covariance analysis of both animals resulted in a correlation coefficient of 0.9908 (Figure 3). Each artificial respiration was also recorded by the telelmery equipment (Positive pressure) accurately as a breath. Both animals responded to the artificial ventilation (bagging) at rates of 15, 10 and 5 breaths per minute with varying degrees of apnea. This apnea was represented by a flat derived respiration line and no breaths were marked. Pressure changes that occurred during bagging and the beginning of the subsequent apnea are shown in Figure 4. The waveform produced by a continued lack of respirations after ventilating the animal is shown in Figure 5. When animals were given Dopram there was a rapid increase in respiratory rate. The increase in Bpm after Dopram was transient and was not easily captured in the 30-minute respiratory rate counts. Direct observation of the breaths and the computer screen confirmed that the algorithm was detecting the increased Bpm. The Epi infusion did induce an increase in respiratory rate that was more sustained. The tracing from an animal breathing over 40 breaths per minute during the Epi infusion showed a clear respiration rate waveform (Figure 6). Sodium pentobarbital and phenytoin in the euthanasia solution depressed respiratory function. Examples of 8 then 4 breaths per minute after euthanasia solution are seen in Figures 7 and 8 respectively. Again the waveforms are distinct in the tracing and marked by the software.

Overall, the P3 respiratory rate software performed very well at the predefined settings in sedated cynomolgus monkeys. The next step was to evaluate the software with actual counts in awake, free moving animals.

Conscious comparison: The continuous collection of data for one week reveled a consistent diurnal rhythm in all parameters measured. This included body temperature and respiration rate, which both increased in the morning and decreased at night (Figure 9). Typical respiratory waveforms from animals during the morning or night data collections are shown in Figures 10 and 11.

DISCUSSION

The respiration module for the P3 analysis module was utilized to evaluate the respiration imposed oscillations on blood pressure waveform from telemetry animals. Waveforms were monitored and collected under different physiological conditions to optimize the software settings. Criteria were chosen that allowed us to measure respiration rates in cynomolgus monkeys over a wide range of conditions and respiratory rates. This study demonstrated that with these settings, the telemetry system was able to accurately measure mechanical ventilation, hyperventilation, apnea, shallow respiration and transitory increase in respiratory rate due to increased body temperature, Dopram V® or Epinephrine infusion.

The overall correlation coefficient between observed respirations and derived respirations for this study was r=0.95. During apnea episodes following mechanical hyperventilation, heart rate and blood pressure remained constant but the respiration tracing of the telemetry system went flat and neither the observer nor the telemetry system counted any breaths. The P3 system display of the respirations was slightly delayed but it was easy to track each respiration as it took place by looking across the animal and viewing the P3 display screen at the same time. Thus we were able to demonstrate that in sedated animals, the telemetry system can accurately monitor respiration at both high (50 Breath Per Minute (BPM)) and low respiratory rates (2 BPM) including apnea.

The data in caged animals is antidotal but the correlation between respiratory rate and body temperature is consistent with known mechanisms. During measurements of animals in their home cages both the respiration waveforms and the derived data show an increase in respiration rates when a person enters the room or the animals are handled. Increased body temperature can only be produced by increased oxidation, which would translate into higher respiration rates. Therefore, for this limited range, the software appears to accurately reflect predicted physiological phenomenon in conscious animals.

CONCLUSION

SNBL USA's Telemetry System is able to consistently and accurately detect and display respiratory rate derived from blood pressure over expected physiological and pathological ranges in an actual animal test system. These values are consistent with values measured by conventional breath counting methods in both sedated and awake cynomolgus monkeys. Therefore, SNBL USA's Telemetry System has proven itself to be a highly useful tool for the evaluation of respiratory rate in Safety Pharmacology studies.

ACKNOWLEDGEMENTS

The authors would like to gratefully acknowledge the technical assistance and contributions of

Dr. Cathy Johnson-Delaney DVM, Dipl ABVP avian, Liane Tetreault, Doug Hasund, Lisa Nydegger, Steve Flores, Linda Cole, Ray Colby, Alisa Mendenhall and Dr. Larry P. Tilley DVM, DACVIM.

Figure 1. Animal No. 1 Respiratory Rate Comparisons

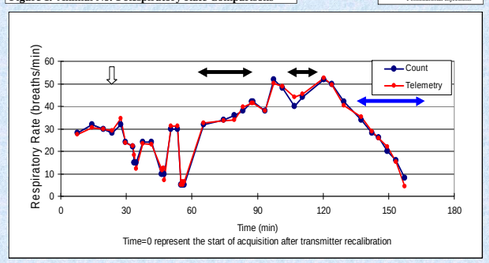

Figure 2. Animal No. 2 Respiratory Rate Comparisons

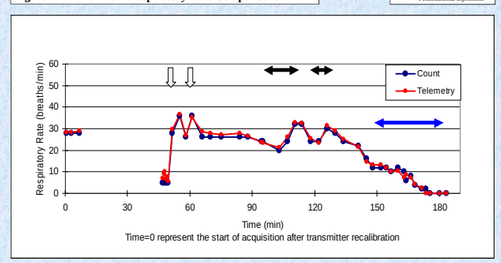

Figure 3. Correlation

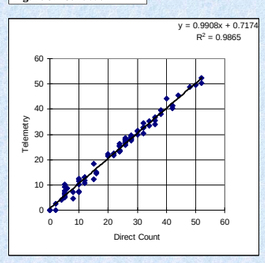

Figure 9. Diurnal Rhythm of Respiratory Rate and Body Temperature in Cynomolgus Monkeys