

The influence of manipulative stress on cardiopulmonary changes in New Zealand White rabbits

Dorota Róžańska¹, Paweł Róžański², Adam Brodzki¹

¹ Department and Clinic of Animal Surgery, Faculty of Veterinary Medicine, Agricultural University, Lublin, Poland

² Department of Animal and Environmental Hygiene, Faculty of Biology and Animal Breeding, Agricultural University, Lublin, Poland

Abstract: The objective of the study was to determine the influence of stress reaction, related to sudden changes of environmental conditions and clinical examination, on cardiac and respiratory function in New Zealand White rabbits. The results measured during clinical examination were compared with values measured after sedation, and with values recognized as norms (physiological range) characteristic for rabbit species. Forty rabbits were sedated with a combination of medetomidine, midazolam and atropine. Before and 5 min. after sedation, rectal temperature, heart and respiratory rates, arterial blood pressures and blood gas parameters were measured. Stress reaction caused by clinical examination evoked a significant increase in respiratory rate. It decreased and achieved physiological range after sedation. There was noted a slight, but statistically significant decrease in systolic arterial pressure after medetomidine-midazolam-atropine (MMA) administration. Significant increase in carbon dioxide pressure (PaCO₂) and statistically significant decrease in arterial oxygen pressure (PaO₂) 5 min. after drugs administration were noted. Results suggest that manipulative stress disturbs the function of the cardiopulmonary systems in rabbits. Measurements obtained in stressed-out rabbits cannot be considered as physiological. Most of cardiopulmonary variables should be measured after sedation, which shows their real, physiological range characteristic for healthy and calm animals.

Key words: rabbits, manipulative stress, cardiopulmonary changes

INTRODUCTION

There are a number of uses for the domestic rabbit. Rabbits are suppliers of healthy and valuable meat, skins and angora wool [1, 2, 3]. In medicine and veterinary medicine they provide valuable experimental material for laboratory, biological and pharmacological tests, as well as for diagnostic investigations [1, 4, 5]. Because of their economic usefulness rabbit husbandry is an important and promising direction of animal production [6]. However, rabbits belong to relatively poorly disease-resistant and sensitive animals [2, 7]. It is generally considered that they are particularly susceptible to stress; therefore, while conducting research on rabbits one should proceed very carefully [3, 5, 8, 9]. Stress is the result of homeostasis disorder and appearing of exhaustion and destruction processes in the organism [6]. Genetic stress immunity is a characteristic which is highly hereditary, and negatively correlated with the useful features most of farm animals, as confirmed in rabbits. It is difficult to breed animals that are both resistant to stress and have highly useful features. The present-day farm animal species are directed to high productivity, but simultaneously they are very susceptible to changes in living conditions.

In spite of progresses in the introduction of modern industrial and experimental technologies, stress reactions

remain constantly present in laboratory animals. Particularly stressful for rabbits are transportation and changes of living conditions [6, 7, 10]. An additional stress factor is clinical examination, which is standard procedure in most experiments. This produces strong psychical stimulation, aggression, and disturbance of homeostasis of the organism, which manifest as cardiopulmonary disorders. Finally, it is difficult to determine whether these changes were evoked by stress or the experiment itself.

The subjects of our study were young New Zealand White rabbits. Little published information is available on reference intervals for blood gas and acid-base constituents, as obtained by routinely available clinical chemical methods in rabbits.

The aim of the study was to estimate the influence of stress reaction, related with sudden change of living conditions and clinical examination, on the function of the cardiac and respiratory systems in New Zealand White rabbits. We compared the values of parameters measured during clinical examination and the values of parameters measured after sedation with the combination of medetomidine-midazolam-atropine, with the values recognized as norms (physiological range) characteristic for rabbit species.

MATERIALS AND METHODS

Experimental animals: 26 adult male and 14 adult female New Zealand White rabbits weighing 3.0-4.5 kg were obtained from a commercial breeder. They were housed individually in stainless steel, wire-bottom cages. The animals were provided

Corresponding author: Dr. Dorota Róžańska, Faculty of Veterinary Medicine, Department and Clinic of Animal Surgery, Agricultural University, Głęboka 28, 20-950 Lublin, Poland.

E-mail: d-rozanska@tlen.pl

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with standard rabbit diet and water available *ad libitum*. All rabbits were examined twice daily. Before the research procedure, the rabbits underwent 14 days acclimatisation to the new environment. Food was withheld at least 12 h prior to each experiment. The study was conducted in accordance with guidelines provided by the Local Ethical Committee of the Agricultural University in Lublin.

Each rabbit was sedated by intramuscular administration of a combination of medetomidine (0.2 mg/kg), midazolam (0.5 mg/kg) and atropine (0.5 mg/kg). All drugs were mixed in a single syringe for injection. Loss of righting reflex was used to measure the duration of sleep time. The righting reflex was evaluated by noting the time interval at which the animal attempted to right itself from dorsal to sternal recumbency.

Local anesthetic cream (Lignocainum hydrochloricum 2% A gel, Jelfa, Poland) was applied to the rabbits' ears 45 min. before administration of anaesthetic to prevent pain during catheter placement [10, 11]. An "over-the-needle" catheter (Becton Dickinson Vacutainer 7/10 38 mm 22G, Poland) was placed in the central ear artery (*a. auricularis intermedia*) and connected to a pressure transducer (Becton Dickinson, Gabarith™ PMSET 1 DT-XX, Poland) to monitor arterial blood pressure. The catheter was secured with adhesive tape.

After placement of the catheter, baseline (t=0) recordings of rectal temperature, respiratory rate, heart rate, systolic and diastolic arterial pressure, were obtained. The rectal temperature was obtained by inserting an electronic thermometer probe 4 cm into the rectum. An arterial blood sample (0.5-1.0 ml) was then obtained for blood gas analysis (t=0 minutes). After clinical examination and the above-mentioned mentioned measurements, which lasted about 30-35 min., the animals were administered intramuscularly the anesthetic combination. Further measurements of rectal temperature, heart and respiration rates were taken after sedation – ca. 5 min. after injection of the mixture. Arterial blood samples determination of arterial PaCO₂, PaO₂, SpO₂, pH, acid-base excess (ABE) and bicarbonate concentration were also collected after sedation. These values were determined with a blood gas analyzer (Corning 328), and corrected automatically to rectal temperature measured at the same time.

Statistical analyses. The values measured in each of the 40 rabbits at each time point were analysed statistically by Statistica 6.0 programme with the use the Wilcoxon test. Wilcoxon test was used to compare the values measured 5 min.

after injection of the drugs with the values measured before their administration (t=0). A value of P<0.05 was considered significant.

RESULTS

Temperature and respiratory rate: The temperature did not change significantly during the experiment (Table 2). It came within the upper physiological limit. The difference between baseline of respiratory rate and the mean values 5 min. after drugs administration was statistically significant (Table 1). Before administration of the drugs, the respiratory rate was much higher than the physiological range. After sedation, it decreased and achieved the upper limit of the physiological range.

Heart rate and blood pressure: Heart rate during the experiment slightly decreased, but the changes were significantly different. However, it came within the physiological range. After sedation, there was a statistically significant decrease in systolic arterial pressure (SAP) compared with baseline. The systolic arterial pressure did not reach the lower physiological level. There were no statistically significant changes in diastolic arterial pressure (Table 2). DAP came within the physiological range.

Arterial blood pH and oxygen saturation: Arterial pH decreased significantly during examinations, but measured values came within the physiological range (Table 1). The SpO₂ value decreased significantly after sedation (Table 1). The baseline of oxygen saturation was physiological, but after drugs administration it was lower than the physiological range.

Blood gas analysis: A statistically significant increase in carbon dioxide pressure (PaCO₂) was observed after sedation. Both values – measured before and after sedation – were within the physiological range. There was a marked, statistically significant decrease in arterial oxygen (PaO₂) 5 min. after administration of the drugs (Table 1). The value measured after drugs administration was lower than the physiological range.

Effect on nociception: All rabbits had positive responses to the stimuli before anaesthesia. All animals were deeply sedated 5 min. after MMA administration, which was suitable for the safe, painless and comfortable taking of measurements. Anaesthesia was administered without complications.

Table 1 Blood gas and acid-base variables before (time 0 min) and after medetomidine-midazolam-atropine mixture administration (time 5 min) compared with physiological range (norm)

	pH	PaCO ₂ (mmHg)	PaO ₂ (mmHg)	SpO ₂ (%)	HCO ₃ ⁻ (mmol/L)	ABE (mmol/L)
Time: 0 min	7.42±0.04	28.47±2.80	83.26±8.96	96.68±0.60	21.23±1.87	4.92±1.75
Time: 5 min	7.38±0.03(**)	33.53±4.19(**)	63.47±13.51(**)	88.52±4.32(***)	21.34±1.87(-)	4.49±2.54(-)
Norm	7.41 ¹¹	33.3 ¹¹	107 ¹¹	93.4 ¹¹	28 ⁴	3.0-5.0 ¹⁴
	7.21-7.57 ¹⁴	22-51 ¹⁴	81.70-89.20 ¹⁴	90-100 ¹⁴	22.3 ¹¹	0.6-6.1 ¹⁵
	7.43-7.49 ¹⁵	21-30 ¹⁷	100-120 ¹⁷	97 ¹⁵	18.40-21.50 ¹⁴	
				86-88 ¹⁷		

Values reported are mean ± SEM (range).

statistically insignificant: – P>0.05;

statistically significant: * P<0.05, ** P<0.01, *** P<0.001.

ABE = Acid-base excess. SpO₂ = Oxygen saturation.

Table 2 Respiratory rate, heart rate, rectal temperature, arterial blood pressures before (time: 0 min) and after medetomidine-midazolam-atropine mixture administration (time: 5 min) compared with physiological range (norm)

	Respiratory rate (breaths/min)	Heart rate (beats/min)	Rectal temperature (°C)	SAP (mmHg)	DAP (mmHg)
Time: 0 min	150.16±16.15	223.47±33.05	39.43±0.40	79.11±4.18	59.42±3.47
Time: 5 min	72.58±22.92(***)	209.68±22.89(*)	39.48±0.45(-)	74.21±4.93(**)	60.95±6.74(-)
Norm	50-60 ²	120-150 ²	38.5-39.0 ²	95-130 ¹²	55-95 ¹⁴
	130-150 ¹¹	170-220 ¹¹	38.0-39.5 ¹²	85-150 ¹⁴	60-90 ¹²
	42-72 ¹²	120-200 ¹²	38.4-39.0 ¹⁴		
	32-53 ¹⁴	260-290 ¹⁴	39.5 ¹⁸		
	80-150 ¹⁷	250-300 ¹⁷			
	32-60 ¹⁸	300 ¹⁸			

Values reported are mean ± SEM (range).
 statistically insignificant: – P>0.005;
 statistically significant: * P<0.05, ** P<0.01, *** P<0.001.
 SAP = Systolic arterial pressure. DAP = Diastolic arterial pressure.

DISCUSSION

Stress reactions occur in every living organism. Short-term and non-invasive stress stimulation causes physiological and metabolic disturbances which usually recover after a few hours. Stress has either a profitable or harmful influence on the organism of animals. If exacerbation and time of its duration do not cross the limits of physiological norms, the effect is profitable. The activation of many organs and systems effect the desirable adaptation, and even strengthen the immune system, as observed, for example, during hardening [6]. Long-lasting and/or intensified stress in individuals with poor adaptable abilities usually leads to decrease of general immunity, decrease of productivity, enlarged morbidity, and even death. Stress is often observed in rabbits with poor adaptive abilities, high susceptibility to change of environmental conditions, and also in large rabbit breeding farms [17]. At the time of catching, restraining, and conducting the clinical examinations, particular areas in the spinal cord and in the subcortical nuclei are stimulated. This provokes the production of emotional type stressors [6]. The symptoms of their release are anxiety and fear. With the exception of behavioural disorders, stress causes disorders in the functioning of many organs and systems, including the cardiac and respiratory systems.

In the presented study, the most essential changes of determined parameters with respect to physiological range (norm), were observed in the respiratory rate (Table 1). Many authors have published that respiratory rate range is about 32-72 breaths per minute in calm animals [2, 3, 12, 13]. The respiratory rate can be measured by a non-invasive method: by observing the chest movement of the animals, without direct contact with them [14]. In our study, the respiratory rate was estimated in a dispensary, after the transportation which activated manipulative stress in the rabbits. It is generally considered that because of the significant susceptibility and timidity of rabbits in stressful situations, e.g. while under restraint, this leads to a substantial acceleration of the respiratory rate [3, 13]. One should therefore consider that the measurements obtained before anaesthesia cannot be considered synonymous with the physiological range. Respiratory rate at the time of clinical examination was very difficult to count (ca. 150/minute), which confirmed the results obtained by other authors [15, 16]. After sedation,

it was about 72/minute, Therefore coming within the upper limits of the physiological range.

Five minutes after MMA mixture administration there was a slight, statistically insignificant increase of rectal temperature (Table 1). Heart rate decreased (P<0.05), but before and after drugs administration it came within the physiological range provided for the rabbit species [15, 16]. However, one should note that many authors establish significantly divergent physiological ranges for heart rate [2, 3, 12, 13]. Some consider 210 beats/min. as tachycardia [2, 12]. According to others, the value of HR indicates bradycardia [3, 13]. In considering the described divergence of opinion, it is impossible to evaluate whether the values measured during the experiment were physiological or not. The lack of significant increase in heart rate during clinical examination, with reference to physiological range, may result from the fact that the norms characteristic for rabbits were probably established in the restrained animals. However, it is interesting that in rabbits examined by the telemetric method [14] the value of heart rate was similar to HR measured before anaesthesia in the reported study. The experiment described concerned rabbits living in the wild. It is possible that in this group of animals the range of physiological parameters differs from those characteristic for domesticated species.

In study reported here, the administration of a combination of medetomidine-midazolam-atropine induced a slight, but significant decrease in arterial blood pH, and a slight but significant increase in carbon dioxide pressure (PaCO₂). This was probably the result of slight respiratory depression caused by the administered drugs [9, 13]. However, both variables were within the physiological range. Changes in acid-base excess and bicarbonate concentrations were not significant. It should be emphasised that the variation in acid-base balance variables may be the result of the application of the tranquilizers which, to various degrees, disturb the function of the cardiopulmonary system in rabbits, as well as other animals [9]. The results of acid-base balance parameters after sedation came within the range characteristic for the rabbit species, and were similar to the values obtained before MMA administration. It is believed therefore, that manipulative stress does not significantly disturb the acid-base balance of arterial blood in rabbits. The parameters of arterial blood oxygenation (Table 2) changed significantly. During the examination, arterial oxygen pressure (PaO₂) decreased

below the physiological limit [13, 15, 16]. It is possible that such a decrease was caused by substantial racial sensibility in the examined rabbits [17, 18, 19]. The oxygen saturation (SpO_2) came within the physiological range before sedation. After sedation, arterial oxygen pressure and saturation SpO_2 significantly decreased. The substantial decrease of arterial oxygen pressure (hypoxia) after MMA combination administration, without simultaneous increase of $PaCO_2$ (hypercapnia), may be the result of alveolar collapse caused by the anaesthetics, and impairment in the exchange of arterial gases at the alveolar level [20]. Arterial blood for acid-base balance and arterial oxygenation analysis was taken after restraining of the animals. Although placing the catheter in the central ear artery and taking blood samples is painless (because of the use of local anaesthetics), the catching and restraining causes anxiety which results in stress reaction. After sedation, the arterial systolic and diastolic blood pressure insensibly decreased. Throughout the entire period of research the pressure was somewhat below or within the physiological range (Table 2). Hypertension was not observed in the examined animals. However, the mechanism for the origin of such a reaction in timid rabbits has not been explained.

CONCLUSIONS

We conclude that the clinical examination of New Zealand White rabbits caused stress reaction, and in effect especially disturbed the function of the respiratory systems. As a result of manipulative stress, it caused a considerable increase in the respiratory rate in the examined animals. After sedation, this value quickly returned to within the physiological range. The presented study shows that the physiological range cannot be established in restrained or stressed-out rabbits, because in most cases it results in demonstrating manipulative stress symptoms. In the case of rabbits, strong stressing-out stimuli are simple procedures: moving from the cages, measurement of rectal temperature, taking arterial blood samples, as well as the measurement of arterial blood pressure. The usefulness of the ranges generally considered as physiological is questionable.

Medetomidine-midazolam-atropine sedation is simple and easy to perform and can be used in studies where clinical examination as well as the measurement of clinical parameters are conducted. This sedation scheme assures the elimination of stress reaction on the results obtained during experiments.

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