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Original research

Stability of unilaterally adrenalectomized rats to anaesthesia using xylazine-ketamine as a model

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ABSTRACT

The stability of every patient to anaesthesia is crucial to a successful surgery and full recovery. This study evaluated the stability of unilaterally adrenalectomized rats to anaesthesia using Xylazine-Ketamine as a model. Twelve female Wistar rats were randomly assigned to two groups of 6 each. Group 1 were the sham group which underwent laparotomy only, while group 2 had their left adrenal glands excised. Three weeks post-adrenalectomy, the animals in both groups were anaesthetized according to standard technique. Anaesthetic parameters assessed include the induction time, time to attain surgical plane of anaesthesia, duration of surgical plane of anaesthesia, duration of anaesthesia, rectal temperature and respiratory rate. Results showed that the mean induction time, time to attain surgical plane of anaesthesia and duration of anaesthesia were not significantly different ($p>0.05$) between the two groups. The pedal withdrawal reflex (PWR) and the whiskers movement (WM) were used to evaluate the duration of surgical plane of anaesthesia. The duration of surgical plane of anaesthesia determined by the PWR was significantly ($p\leq0.05$) higher in the unilaterally adrenalectomized rats. There were also significant decreases in rectal temperature and respiratory rate when the unilaterally adrenalectomized groups were compared to the controls. From the results, the unilaterally adrenalectomized rats were comparatively as stable to Xylazine-Ketamine anaesthetic protocol as their intact counterparts, at three weeks post-adrenalectomy. However, there may be need to exercise caution when choosing an anaesthetic protocol for unilaterally adrenalectomized animals with special considerations given the depth and duration of anaesthesia required for a procedure and the length of time post-adrenalectomy.

KeyWords: Anaesthesia, rats, stability, unilateral adrenalectomy, Xylazine-Ketamine.

INTRODUCTION

The adrenal glands, also known as the suprarenal glands, are bilaterally symmetrical endocrine glands located anterior to the kidneys over the medial aspect of the cranial poles of each kidney. The glands have two anatomically and physiologically distinct parts; the outer medulla and the inner cortex (Tischler & DeLellis, 1988). The cortex is mesodermal in origin and secretes cortisol, corticosterone, and aldosterone, whereas the medulla originates from the ectoderm and produces epinephrine, norepinephrine, and dopamine (Sejian *et al*., 2008).

Many diseases of the adrenal glands affect man and animals and include Cushing's syndrome (hyperaldosteronism), Addison's disease (hypoaldosteronism) and chromaffin cell tumours (Pheocromocytoma) (Gorman, 2013). Cushing's disease accounts for more than 70% of cases in adult humans and about 60-70% of cases in children and adolescents (American Association of Neurological Surgeons, 2003). These disease conditions are treated either chemotherapeutically or surgically.

Adrenalectomy which may be unilateral or bilateral is the intervention of choice for the above-stated adrenal diseases. Unilateral adrenalectomy causes temporary adrenal insufficiency as it takes a while for the remaining adrenal gland to compensate and restore the body's homeostasis (Fossum, 2013). For surgical interventions, anaesthesia is an indispensible and most crucial pre-requisite in both humans and animals, providing safety and maximum performance for the surgeon (Muhammad *et al*., 2009).

According to Lang *et al*. (2011), perioperative mortality rates for adrenalectomies have been documented as 6% for elective procedures increasing to 50% for emergencies. This necessitates an urgent need to evaluate the stability of adrenalectomized animals to anaesthesia considering that even intact animals are associated with high level of surgical risk. The stability of the unilaterally adrenalectomized

patients to general anaesthesia is very crucial since unilaterally adrenalectomized animals still lead a normal quality life (Velema *et al*., 2018), and can be exposed to conditions that may warrant other surgical interventions, and invariably, anaesthesia.

Anaesthetic indices are indicators of homeostasis as patient's adequate response to anaesthesia is a good indicator of proper circulatory adjustments to maintain homeostasis (Price, 1960). This throws more light on the stability of the patient, which reflects the body's ability to return to normal homeostasis following a surgical intervention including unilateral adrenalectomy. A stable patient has minimal anaesthetic risk.

Xylazine, a sedative is used as a premedicant and may induce cardiovascular and respiratory depression in patients (Fayed *et al*., 1989). Ketamine, used for both induction and maintenance of general anaesthesia, is known to preserve respiratory drive, and its sympathomimetic properties result in an increase in blood pressure and heart rate (Golzari *et al*., 2013). When used as an anaesthetic protocol, the combination produces adequate surgical anaesthesia in patients, and balances their individual undesirable effects (Ozkan *et al*., 2010).

The objective of the present study was to evaluate the response and stability of unilaterally adrenalectomized rats to anaesthesia through measurement of anaesthetic indices and other physiologic parameters under Xylazine-Ketamine anaesthetic protocol.

MATERIALS AND METHODS

EXPERIMENTAL ANIMALS AND MANAGEMENT

Twelve (12) female Wistar rats with a mean body weight of 217.1 ± 4.6 g, were used for this experiment. The rats were sourced from the Faculty of Veterinary Medicine, University of Nigeria Nsukka, Enugu State. They were housed in stainless steel cages at room temperature in clean well ventilated, fly-proof animal houses in the Department of Veterinary Surgery and Radiology, Michael Okpara University of Agriculture Umudike, Abia State. The rats were allowed to acclimatize for 1 week and fed standard feed (Chikun® Finisher, Nigeria). Water was provided *ad libitum* all through the course of the experiment. Ethical approval was obtained from the University Ethics Committee (MOUAU/CVM/REC/202302).

UNILATERAL ADRENALECTOMY

The rats were randomly assigned to two groups of 6 rats each as follows. Group 1 rats were the sham group (Laparotomy only) while the group 2 had their left adrenal gland removed (UA). Xylazine (VMD, Arendonk-Belgium) (5 mg/kg, IM) was administered to the rats as premedicant, followed by the administration of Ketamine (Ketamine hydrochloride injection USP® Rotexmedica, Germany) (35 mg/kg, IM), 10 minutes after. The paracostal approach described by Fossum (2013) was used to carry out the unilateral adrenalectomy, though the glands were removed intact without blood vessel ligation.

ANAESTHETIC PROTOCOL

Three (3) weeks post-surgery, the animals were placed under general anaesthesia using Xylazine-Ketamine protocol at the same dose as was used for the unilateral adrenalectomy earlier described above.

DATA COLLECTION

The animals were observed throughout the duration of anaesthesia, and anaesthetic indices (induction time, time to attain surgical plane of anaesthesia, duration of surgical plane of anaesthesia and duration of anaesthesia) and some physiologic parameters (respiratory rate and rectal temperature) were monitored to assess the stability of the animals. The time of induction is the time difference (minutes) from the time of injection of the anaesthetic to loss of righting reflex. The time to attain surgical plane of anaesthesia is the time difference (minutes) from the time of injection to loss of pedal withdrawal reflex. The duration of surgical plane of anaesthesia is the time difference (minutes) from the loss of pedal withdrawal reflex to the return of pedal withdrawal reflex. The time difference (minutes) from the loss of whiskers movement to restoration of mild whiskers movement was also used to assess the duration of surgical plane of anaesthesia. Also, the time difference (minutes) from the return of mild whiskers movement to vigorous whiskers movement indicated a period of lightening of anaesthesia. The duration of anaesthesia is the time difference (minutes) between loss of righting reflex to return of righting reflex. The respiratory rate (breathe/minute) and rectal temperature (°C) were monitored every 10 minutes over a period of 160 minutes.

DATA ANALYSIS

Statistical analysis was performed using SPSS software® (Version 20 for Windows, SPSS Inc., Chicago, USA). The data were presented as mean ± standard error of mean (SEM) and analyzed using Student t test statistic and $p<0.05$ was considered significant.

RESULTS

ANAESTHETIC INDICES

The results of the effect of unilateral adrenalectomy (UA) on anaesthetic indices are presented in Figures I-VI. A smooth induction of anaesthesia was observed in both groups with the mean induction time in the sham-operated group (2.00±0.32) not differing significantly ($p>0.05$) when compared to the unilaterally adrenalectomized group (3.40±0.98) (Figure I).

The time to attain surgical plane of anaesthesia in the sham-operated group was 11.40±3.5 minutes. This increased to 15.00±5.65 minutes in the unilaterally adrenalectomized rats. However, there was no significant difference between the mean induction times in the two groups (Figure II).

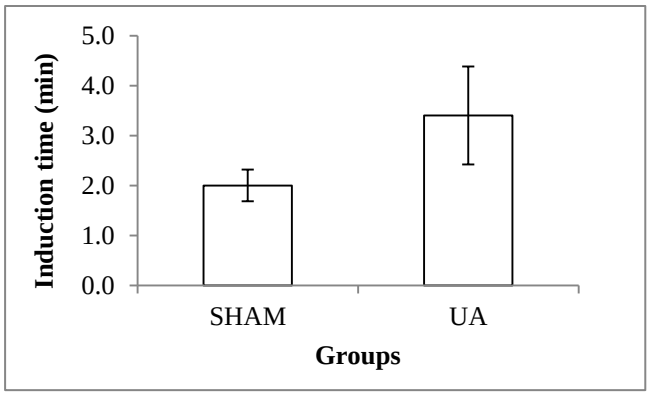

Figure I: Effect of unilateral adrenalectomy on the induction time. *There was no significant difference in the induction time of control sham rats and unilaterally adrenalectomized (UA) rats.*

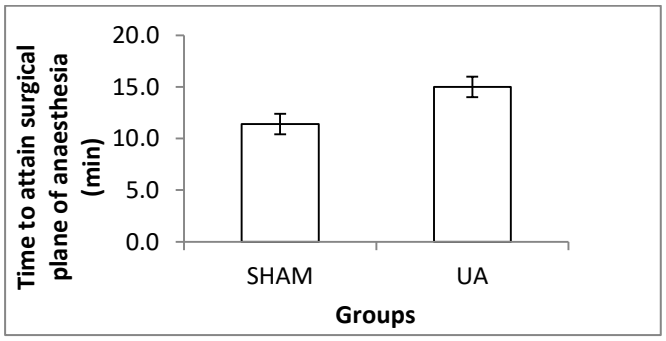

Figure II: Effect of unilateral adrenalectomy on the time to attain surgical plane of anaesthesia. *There was no significant difference in the time to attain surgical plane of anaesthsia of control sham rats and unilaterally adrenalectomized (UA) rats.*

The pedal withdrawal reflex and the whiskers movement were used to evaluate the duration of surgical plane of anaesthesia. The duration of surgical plane of anaesthesia determined by the pedal withdrawal reflex was significantly ($p \leq 0.05$) higher in the unilaterally adrenalectomized rats (154.50±5.42) when compared to the sham-operated group (92.00±14.92) (Figure III). The duration of surgical plane of anaesthesia determined with the whiskers movement (Figure IV) was 79.00±16.16 minutes in sham-operated group and 104.00±21.35 minutes in the unilaterally adrenalectomized group but the difference was not statistically significant ($p>0.05$). The result of the whiskers movement also showed a period of lightened anaesthesia (Figure V) which was 61.00±14.87 minutes in the sham-operated rats and 53.00±13.29 minutes in the unilaterally adrenalectomized rats. This decrease observed was also not significantly ($p>0.05$) different when the two groups were compared.

The duration of anaesthesia which was 309.00±18.23 minutes in the sham-operated group and 328.20±26.62 minutes in the unilaterally adrenalectomized was also not significantly ($p>0.05$) different when both groups were compared (Figure VI).

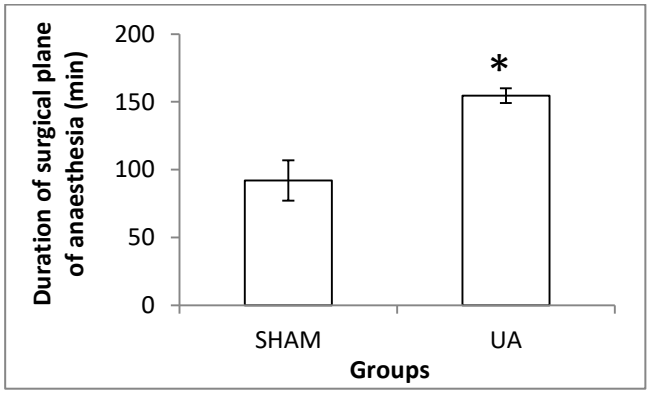

Figure III: Effect of unilateral adrenalectomy on the duration of surgical plane of anaesthesia determined by pedal withdrawal reflex. **There was a significant ($p<0.05$) increase in the duration of surgical plane of anaesthesia when the unilaterally adrenalectomized (UA) rats was compared to control (group I).*

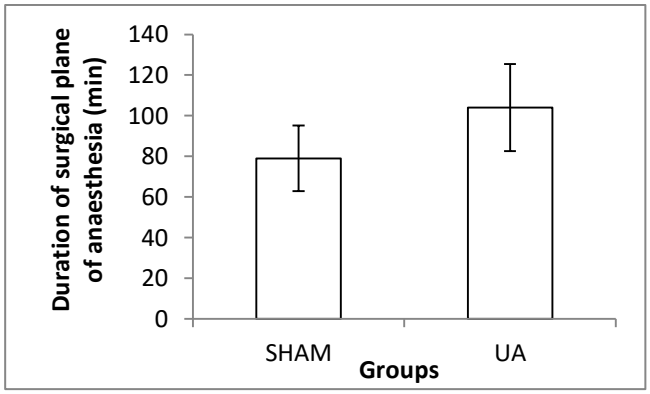

Figure IV: Effect of unilateral adrenalectomy on the duration of surgical plane of anaesthesia determined with whiskers movement. *There was no significant difference in the induction time of control sham rats and unilaterally adrenalectomized (UA) rats.*

PHYSIOLOGICAL PARAMETERS

Figure VII, a graphical representation of the effect of unilateral adrenalectomy on the body temperature, showed no significant difference in the rectal temperature between the two groups from the 10^{th} to the 80^{th} minute. However, there was a significant ($p<0.05$) decrease in the body temperature of the unilaterally adrenalectomized rats from the 90^{th} (32.37±0.12), 100^{th} (32.57±0.09), 110^{th} (32.33±013), 120^{th} (32.27±0.07) to 130^{th} (32.10±0.06) minute when compared to the mean values of the sham-operated rats (33.23±0.24, 33.30±0.20, 33.30±0.10, 33.10±0.00 and 33.13±0.19) respectively.

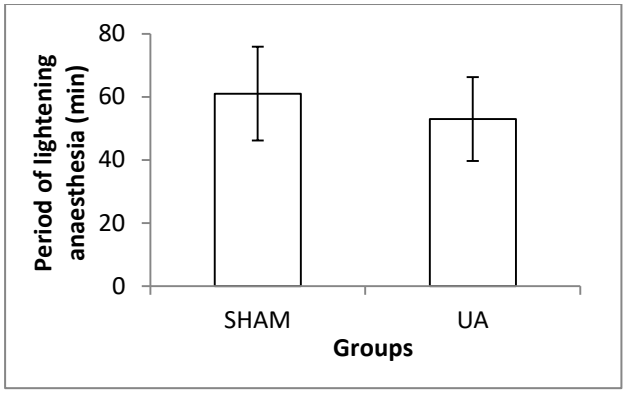

Figure V: Effect of unilateral adrenalectomy on the period of lightening anaesthesia using whiskers movement. *There was no significant difference in the period of lightening anaesthesia of control sham rats and unilaterally adrenalectomized (UA) rats.*

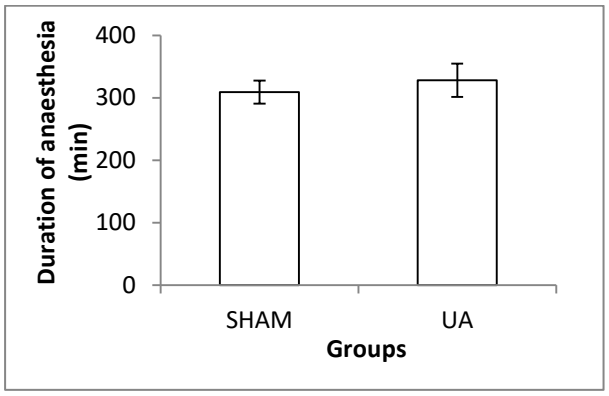

Figure VI: Effect of unilateral adrenalectomy on the duration of anaesthesia in female Wistar rats. *There was no significant difference in the duration of anaesthesia of control sham rats and unilaterally adrenalectomized (UA) rats.*

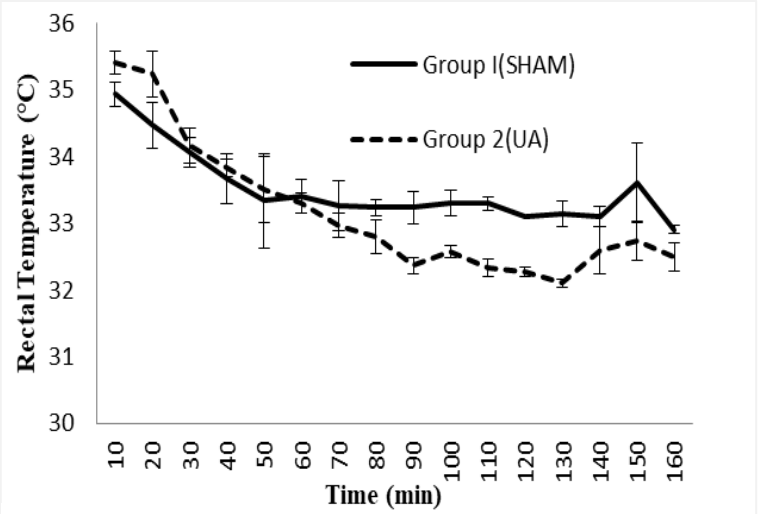

Figure VII. Effect of unilateral adrenalectomy (UA) on the rectal temperature. UA: unilateral adrenalectomised group

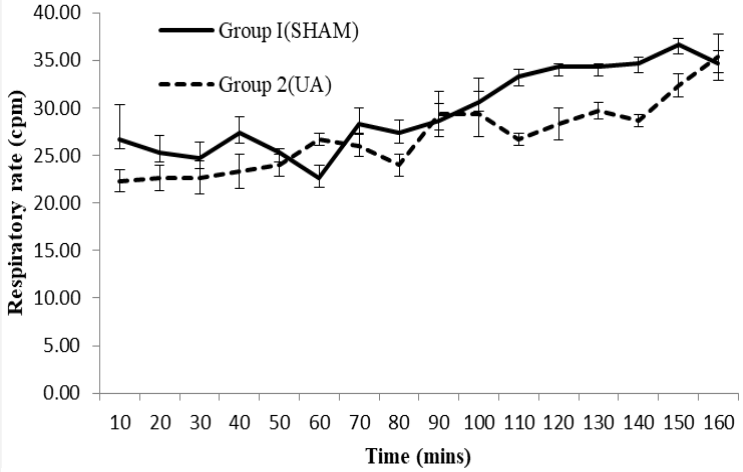

Figure VIII. Effect of unilateral adrenalectomy (UA) on the respiratory rate. UA: unilateral adrenalectomised group

The result of the effect of UA on the respiratory rate (Figure VIII) showed no significant difference from the 10th minutes to the 100th minutes and 160th minutes. But from the 110th (26.67±0.67), 120th (29.33±1.67), 130th (29.67±0.88), 140th (28.67±0.67) to the 150th minute, (32.33±1.20), there was a significant ($p \leq 0.05$) decrease in respiratory rate of the unilaterally adrenalectomized rats when compared to the respective control [(33.30±0.67), (34.33±0.33), ((34.33±0.33), (34.67±0.67), (36.67±0.67)].

DISCUSSION

This study evaluated the impact of unilateral adrenalectomy (UA) on the monitored anaesthetic indices as well as on the body temperature and respiratory rate. The results of the anaesthetic indices showed that the unilaterally adrenalectomized animals were relatively stable. With the exception of duration of the surgical plane of anaesthesia as determined by the pedal withdrawal reflex the monitored anaesthetic indices were comparable with the control. The similarities in induction time and time to attain surgical plane of anaesthesia indicated a similarity in drug metabolism in both groups. The significantly longer duration of surgical plane of anaesthesia seen in the unilaterally adrenalectomized rats may be due to the significantly lowered body temperature observed in this group. Hypothermia increases drug concentration time and thus prolongs drug response (Zhou & Poloyac, 2011).

Also, from this study, the whiskers movement varied slightly with the pedal withdrawal reflex in both the unilaterally adrenalectomized and the sham-operated. Both indices measure the duration of surgical plane of anaesthesia. However, the whiskers movements were regained earlier than the pedal withdrawal reflex in both groups. Although the whiskers movement is a good indicator of anaesthetic

depth (Tandon *et al*., 2008), the loss of pedal withdrawal reflex is the primary indicator of surgical plane of anaesthesia for most laboratory animals (Research Animal Training England, 2020). Their combination could be of anaesthetic monitoring importance, with the whiskers movement serving as an early indicator for anaesthetic top-up when required.

Although there were no significant differences in most of the monitored anaesthetic indices, it was observed that their mean values in the unilaterally adrenalectomized rats were higher than for the sham-operated group. This could be an indication that the remaining adrenal gland may not yet have fully restored the body's metabolic level to the pre-adrenalectomy state.

Physiological parameters are very important indices for the monitoring of anaesthesia. They help in the detection of any abnormalities before they occur and also serve as good indicators for when modifications in anaesthetic permutations to facilitate or maintain smooth and uneventful anaesthesia during surgeries are required. Anaesthesia and surgery can cause rapid changes in vital functions (Standards for Basic Anesthetic Monitoring, 2016). The significantly reduced body temperature and respiratory rate in the adrenalectomized rats compared to the sham-operated occurred during the surgical plane of anaesthesia. This confirms the need to monitor adrenalectomized rats more closely than their intact counterparts.

Generally, the quality of anaesthesia is crucial for the wellbeing and recovery of patients, standardization of experiments, and reduction in anaesthesia-associated morbidity (Alves *et al*., 2010).

In conclusion, though the unilaterally adrenalectomized animals were relatively stable to Xylazine-Ketamine anaesthesia, the selection of anaesthetic protocol for any surgery involving patients with single adrenal gland should be carefully evaluated, particularly with regards to depth and duration of surgical plane of anaesthesia. Consideration should also be given to the length of time post-adrenalectomy in such individuals.

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