DOI: 10.24996/ijs.2018.59.1B.2

ISSN: 0067-2904

Effects of RVLM Stimulation on Blood Pressure and Vasopressin-Like Levels in Brain Sites and Plasma are an Anesthetic Dependent.

Imad Hatim Rashid
Department of Biology, College of Education, Al-Iraqia University, Baghdad, Iraq.

Abstract

The study is sought to determine specific alteration in AVP-LI level of plasma and sites of the hypothalamic-neurohypophysial-system; supraoptic nucleus (SON), paraventricular nucleus (PVN), neurointermediate lobe (NIL), and median eminence (ME), related to effects of stress of different anesthetics and electrical stimulation of RVLM area. Urethane or halothane anesthetized Wistar rats were subjected to intra-RVLM electrical stimulation of 10 sec train, 1msec pulse duration at 40 Hz, and 2V every 30s. After 30min stimulation, AVP-LI extracted from plasma, NIL, ME, SON, and PVN and measured by RIA, were compared with that of sham-stimulated urethane- or halothane-anesthetized rats and unstimulated unanesthetized control rats. Data were expressed as mean ± S.E.M. and analyzed using as appropriate Student's paired or unpaired t-test, and one way ANOVA. The results demonstrates presence of differential effects of the used anesthetics on resting BP, and increased BP to RVLM-stimulation, as well as a differential reduction in AVP-LI level which is significant ($p<0.05$) with urethane in SON NIL, ME, and blood plasma, but not in PVN, which is in contrast to urethane was significant with halothane, suggest a central role for halothane on release of PVN-AVP vs. role for urethane on the release of AVP at the level of hypothalamic-neurohypophysial-system. However, stimulation of RVLM abolished the regional specificity of anesthetics on SON vs PVN-AVP levels, indicated neither anesthetic prevented the central mechanisms involved in the response of AVP neurons to RVLM stimulation. In conclusion, halothane is preferred for examining the effects of RVLM-stimulation on AVP changes, whereas in term of their effects on the pressor response to stimulation of the RVLM area, urethane appears preferable.

Keywords: RVLM/C1, AVP, urethane, halothane, plasma, NIL, ME, SON, PVN.

تاثيرات تحفيز منطقة RVLM/C1 على ضغط الدم ومستويات الفاسوبريسين في بلازما الدم ومناطق مختلفة في الدماغ تتأثر بنوع المخدر

عماد حاتم رشيد
قسم علوم الحياة، كلية التربية، الجامعة العراقية، بغداد، العراق.

الخلاصة

سعت الدراسة الى تحديد التغييرات النوعية في مستوى هرمون الفاسوبريسن (vasopressin) في كل من بلازما الدم وفي مواقع الجهاز الوطائي–النخامي عصبي (neurohypophysial system)، كالنواة فوق البصرية (supraoptic nucleus) والنواة جارة البطين (paraventricular nucleus)، وربوة الوسط (median eminence)، والفص العصبي المتوسط (neurointermediate libe)، التي لها علاقة بآثار

Email: dr_rashid9@yahoo.com

الإجهاد الناجم عن مواد التخدير المختلفة اوالتحفيز الكهربائي لمنطقة RVLM. تعرضت خلال التجربة جرذان وبستر تحت تاثير مخدر اليوريثان (urethane) أو الهالوثان (halothane) إلى تحفيز منطقة RVLM في ساق الدماغ بتيار كهرباء لعشرة ثواني، مدة نبض 1msec على 40 Hz و 2 V كل 30ثانية لمدة 30 دقيقة. بعد التحفيز تم عزل الفاسوبريسين من البلازما ومن مواقع الجهاز الوطائي–النخامي عصبي المذكورة، وقياسه بطريقة التشعيع المناعي (radioimmunoassay)، ومقارنة مستوياته بتلك التي تم تحفيزها تحت تاثير الهالوثان او اليورثان خارج منطقة RVLM، او بمجموعة السيطرة التي لم تاخذ اي تخدير او تحفيز. تم التعبير عن البيانات على أنها تعني ± S.E.M. وتحليلها باستخدام طريقة اختبار– t المقترن (paired Student t–test) أو غير المقترن (unpaired Student t–test) المناسبة، او طريق– واحد أنوفا (one way ANOVA). اظهرت النتائج وجود تاثير تفاضلي لمواد التخدير على ضغط الدم الاساسي (basal) وعلى زيادة ضغط الدم نتيجة تحفيز منطقة RVLM ، فضلا عن انخفاض تفاضلي معنوي في مستوى فاسوبريسين ($p < 0.05$) مجموعة اليوريثان في كل من النواة فوق البصرية والربوة المتوسطة والفص العصبي المتوسط وبلازما الدم، باستثناء النواة جارة البطين التي على عكس ذلك اظهرت في مجموعة الهالوثان انخفاضاً للفاسوبرسين فيها، وهو ما يشير إلى وجود دور للهالوثان في افراز الفاسوبريسين في الدماغ مقابل دور لليوريثان في افراز الفاسوبريسين على مستوى الجهاز الوطائي–النخامي عصبي. ومع ذلك، فإن تحفيز منطقة RVLM ألغت الخصوصية المناطقية لتاثير مواد التخدير على مستويات الفاسوبرسين في النواة فوق البصرية مقابل النواة جارة البطين، مما يعني انعدام اي تاثير للمواد المخدرة على منع الآليات المركزية التي لها دور في استجابة خلايا الفاسوبريسين الى تحفيز RVLM. يستنتج من ذلك ان الهالوثان كان الافضل في اختبار دور تحفيز منطقة RVLM على تغيّرات فاسوبريسين الدماغ، بينما كان اليوريثان الافضل في اختبار استجابة الضغط لتحفيز منطقة RVLM.

Introduction

Studies on the brain circuit between the vasopressin (AVP) producing neurons of hypothalamic, supraoptic nucleus (SON) and paraventricular nucleus (PVN), and the rostral ventrolateral medullary (RVLM) region, revealed a bidirectional connection. Each of SON and PVN have two distinct subdivision of AVP neurons; a) the magnocellular neurons secreting AVP into the peripheral circulation from axon terminals in the neural lobe of the pituitary and acts to maintain body fluid homeostasis and water conserving in the kidney that depends upon osmotic stimulation [1], and b) the parvocellular neurons on one hand projects outside the central nervous system (CNS) to the external zone of the median eminence (ME) from where AVP is secreted into the pituitary portal circulation to maintain electrolyte homeostasis and stress (increases) independent of the osmotic status, and on other hand projects to many sites within the CNS [2].

PVN-parvocellular neurons plays a role in regulating cardiovascular function and sympathetic outflow [3], and an important component of the central neurocircuitry of the cardiac sympathetic afferent reflex (CSAR) [4]. Studies on the parvocellular subdivision provides an anatomical bases for three means of influence by which the PVN can have on sympathetic activity and cardiovascular regulation; projection to the RVLM where the presympathetic motor neurons; a projection running in parallel with this but bypassing the RVLM to the spinal cord where the sympathetic preganglionic neurons; and a branching population innervating neurons in both the RVLM and spinal cord [5, 6, 7]. $V1_A$ and OT receptors appear to be differentially distributed within the RVLM, with AVP $V1_A$ receptors expressed throughout the rostro-caudal axis [8] and OT receptors confined to a small population of neurons in the pre-Botzinger complex [9]. In addition to AVP released from PVN neurons [8], a number of neuronal inputs containing excitatory and inhibitory neurotransmitters also converge on RVLM sympathoexcitatory neurons from multiple areas of the brain and spinal cord to control BP by regulation of SNA; for example, endogenous angiotensin II (AngII) [10] potentiate RVLM neurons, whereas GABAergic inhibitory input from caudal ventrolateral medulla neurons suppresses RVLM neuronal activity [11], and these opposing influences regulate its neuronal excitability. Studies have demonstrated implication of the released neurohormone AVP of hypothalamic SON and PVN, in the RVLM in elevation of mean blood pressure (BP) due to activation of AVP-V_1 receptors [12], which also found to mediate increased BP and plasma hyperviscosity to

microinjection of AVP into the RVLM of normotensive rats [13]. In a very recent study, bilateral microinjection of AVP into the RVLM of Wistar rats, increases BP and sympathetic vasomotor tone, and decreases HR; responses that are abolished after microinjection of the AVP $V1_A$ antagonist SR 49059 in the RVLM. Importantly, microinjection SR 49059 is also effective in blocking the [Pyr^1] apelin-13-induced increases in MABP, HR and sympathetic tone, suggesting that [Pyr^1]apelin-13 acting in the RVLM increases BP and vasomotor tone via a vasopressinergic mechanism [14]. AVP released by a subset of PVN neurons via the AVP V_{1A} receptor may activate neurons located at the lateral VLM- [15], medial VLM and/or rostral ventral respiratory column (rVRC)- to inhibit respiratory–related hypoglossal nerve activity [16], and in RVLM and rVRC to increase sympathetic nerve activity in chronic intermittent hypoxia [17]. Other study demonstrated involvement of a subpopulation of oxytocin of PVN, in cardiovascular (increase BP and HR) and respiratory activity, partially via projections to the RVLM and phrenic nuclei [9].

One possible reciprocal projection of the vasopressor area in the RVLM/C1 region to the hypothalamus is adrenergic pathway [18], using adrenaline (AD) as neurotransmitter. This is supported by the finding that the pressor response induced by electrical stimulation of the rostral VLM/C1 region of the rat is associated with AD release in the posterior hypothalamus, but not NA release [19], and an increase in plasma AVP, AD, and NA [20]. Release of AD from the hypothalamic terminals of the RVLM in the SON and PVN has been confirmed by neuronal transection decreasing the rate of AD release in hypothalamus, indicating that the resting release of this amine is dependent on impulses from the cell body [21].

No previous study however, has demonstrated RVLM stimulation induced parallel changes in AVP levels in the hypothalamic-neurohypophysial-portal system associated with plasma hormone levels. The present investigation sought to determine the effects of 30 min electrical stimulation of the RVLM/C1 region on the level of plasma AVP, and brain AVP levels in NIL, ME, PVN, and SON measured by RIA. The approach used was to electrically stimulate the RVLM/C1 area in anaesthetized animals. A major problem in addition to the anesthetic issue relates to the inevitable effect of stress increasing the level of plasma AVP following major surgery [22], and the use of ketamine anesthesia [23]. Since urethane was the anesthetic of choice in this study, and to avoid involvement of urethane with the effects of RVLM stimulation on AVP release, the results obtained from urethane anaesthetized rats were compared with those obtained from halothane anaesthetized or unanaesthetized/unstimulated rats.

Materials and Methods

The study were performed on male Wister rats (250-300g) maintained under 12h light: 12h dark photoperiod with food and water available ad libitum. The rats were divided into five groups each of 6 as follows: a) controls received sham-stimulation to control area outside the RVLM region, b) treated groups received stimulation to the RVLM area. Both a) and b) were divided into two groups anaesthetized either with halothane or urethane, and c) a control group that received neither anesthetic nor stimulation. Urethane (Sigma) was administrated i.p. at a dose of 1.1g/kg in a volume of 4 ml/kg of a 25% saline w/v solution, and maintenance dose being given when required. Halothane (Fluo-vac, International Market Supply) was administered via a cone placed over the nose with a starting concentration of 3% in a mixture of 50% O2 and 50% NO2, reduced to 2% during the surgical procedure. After surgery the concentration was further reduced to 1% until the end of the experiment.

For blood pressure measurement following anesthesia,, a skin incision over the thigh, exposing blood vessels, facilitated the insertion of a polyethylene catheters (PE100) either connected to a 1 ml syringe containing heparinized (30 units/ml) saline (0.9% NaCl w/v) into the left femoral vein for i.v. injection purposes or connected to a blood pressure transducer into the left femoral artery for recording of BP, via an insertion made with fine femoral artery for recording of BP, via an insertion made with fine scissors. The cannula was tied to the artery or vein and Body temperature was maintained between 36 and 37 C^0 by an appropriate heat source. Systolic and diastolic BP and HR were monitored continuously throughout the experiment, by connecting the catheter fixed to the femoral artery to a blood pressure transducer (type 4-422-0001) with a modified low volume displacement dome (Ardill, Fentem, Hellard, 1968). The catheter was filled with heparinized saline connected, via an amplifier (type SE 4910, Emma) to an ultraviolet oscillograph recorder (3600, SE Laboratories Ltd, England), to record systolic and diastolic BP, while the HR was computed from the pulse wave and displayed on the oscillograph by the instantaneous rate meter (Type 275, Devices instruments Ltd, England).

Thereafter, SBP peak variations to resting and electrical stimulation of the RVLM region were calculated at 5 min intervals to compare the BP magnitudes and reproducibility during the experiment. To implant of stimulating electrode, halothane or urethane anaesthetized rats were mounted in a stereotaxic frame (David Kopf Instrument, Tujunga, CA) following catheterization with the incisor bar set at 3.3 mm above the intraaural line for urethane anaesthetized rats. For the halothane experiments a custom-built integral incisor bar/nose piece was compared to a scavenger unit for adsorption of exhaled halothane. The scalp was incised to expose the skull and cranium then cleaned and a burr hole was drilled in the skull, without piercing the dura. A concentric needle electrostimulator SNE 100 (Clarke Electrochemical Equipment, 100 µm.o.d) connected to the stimulating apparatus (Farnll Physiological Stimulator, England) set to deliver current at 10 sec trains with a pulse duration of 1msec at 40 Hz and 2 volts every 30sec through the electrode which was inserted into the RVLM/C1 region using the following co-ordinates; Rostro-caudal (R.C.) -11.8mm posterior to the bregma, lateral (L) +1.6mm lateral to the midline, and ventral (V) -8.3mm ventral from dura according to the atlas of Paxinos and Watson [24]. To map the site that gave the greatest pressor response, the electrode was moved ±1.0mmrostro-caudal, medio-lateral, and/or dorso-ventral from the used co-ordinates, using tracks separated by 0.2mm in these directions. The final position of the inserted electrode in the most active sites of RVLM/C1 area was determined by finding the area that produced the maximum increase in BP using a single 10sec train of 1msec square wave pulse duration at 40Hz and 2V of electrical stimulus repeated whenever needed Figure-1(A).

In sham-stimulated rats (urethane or halothane anaesthetized control groups), the electrode was positioned in a control area close to but outside the RVLM/C1 region, where a single train of electrical stimulation showed no pressor response Figure-1(B). The electrode remained in position throughout the experiment. The rats were then left at least one hour for the BP to maintain a stable base line before RVLM/C1 stimulation for 30 min, using a 10 sec train of 1msec pulse duration at 40 Hz and 2 V every 30s Figure-2.

For histological verification of stimulating electrode and injecting cannula at the end of each trial and before decapitation the tested animal received, a small electrical lesion using 20V 1ms rectangular pulses at 40Hz for 15 sec in order to mark the site of RVLM/C1 stimulation. The stimulating electrode was removed and the rat immediately killed and decapitated and their blood was collected and brain removed for dissection of brainstem and other regions (see below) as described elsewhere [25] and shown in Figure-(2).

The brainstem was cut on a freezing microtome, in serial coronal sections (40µm), mounted on gelatin covered slides and stained with restyle violet. Sections of the position of the stimulating electrode was verified under a light microscope as seen in Figure-3. Blood sample (~5ml) was collected into heparinized (75 units/ml) coated tubes from the trunk side of the decapitated rat, and centrifuged (3000 rpm, 4 ^{0}C for 15 min) immediately to separate the plasma from the blood cells. The samples was then stored at -80 ^{0}C for subsequent AVP extraction and measurement by RIA. The brain was placed on its basal surface on a cool hard rubber plate. The NIL was separated from the pituitary's two anterior lobes and ME was plucked out of the top of the hypothalamus, then coronal brain slices were cut by hand and immediately further dissected , into the SON and PVN, using the method of Palkovits and Brownstein [26], and as described elsewhere [25].

For extraction of plasma and tissue AVP and measurement by RIA, brain tissue samples were immediately homogenized for AVP extraction by sonication (Ultrasonicator Soniprep 150, England) in 1 ml of 1M acetic acid (Fisons, England). The supernatants containing AVP were separated by centrifugation (4000 rpm for 20 min), dried down in a vortex evaporator (Buchler) and stored at -20 ^{0}C for subsequent measurement by RIA. The pellet was stored at -20 ^{0}C until protein determination [27]. AVP was extracted from plasma by florisil using the technique of Rooke and Baylis [28]. The test of percentage extraction and used antisera specificity and validation for the measurement of tissue and plasma AVP were respectively described elsewhere [25]. Tissue and plasma samples to be measured by RIA were resuspended in RIA buffer and assayed for AVP by RIA using the technique of Jenkins *et al*., [29] as described elsewhere [25].

Data Analysis: Results were expressed as mean ± S.E.M. and n. is the number of the animals. Student's unpaired t-test were used for statistical analysis of the effects of urethane and halothane on the basal and the increase in SBP during RVLM stimulation. Mean values at the time points -6 and -1 min (before stimulation), and 1, 6, 11, 16, 21, and 26 min (during RVLM/C1 stimulation), for urethane

and halothane anaesthetized, and control rats. The AVP level differences, measured by RIA, between urethane and halothane in RVLM-sham stimulated groups were compared to that in RVLM stimulated groups as well as to that in the control group, using analysis of variance (ANOVA).

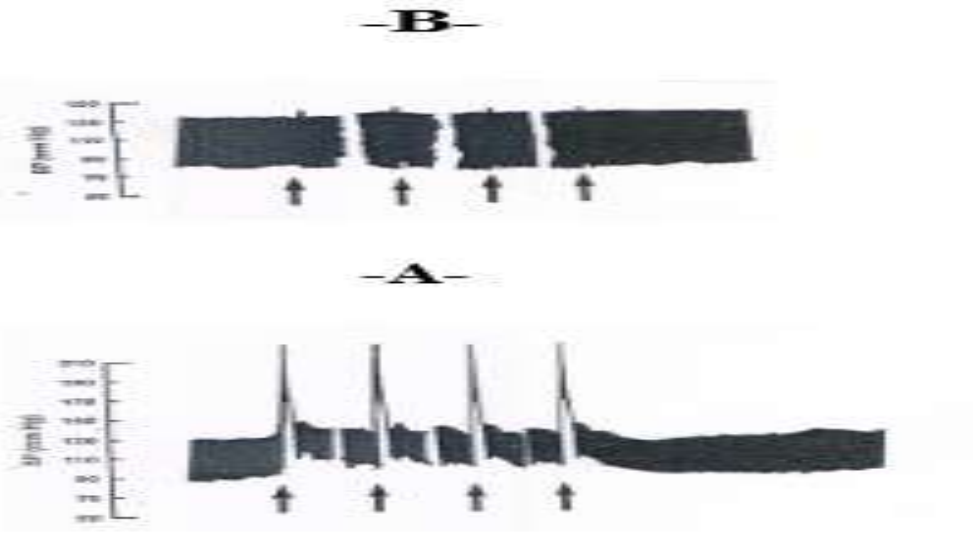

Figure1-Traces demonstrating the SBP responses during a single electrical stimulation (↑) to the RVLM region (A), or to the control areas outside the RVLM region (B), with a 10sec trains of 1msec square wave pulse duration at 40Hz and 2volts.

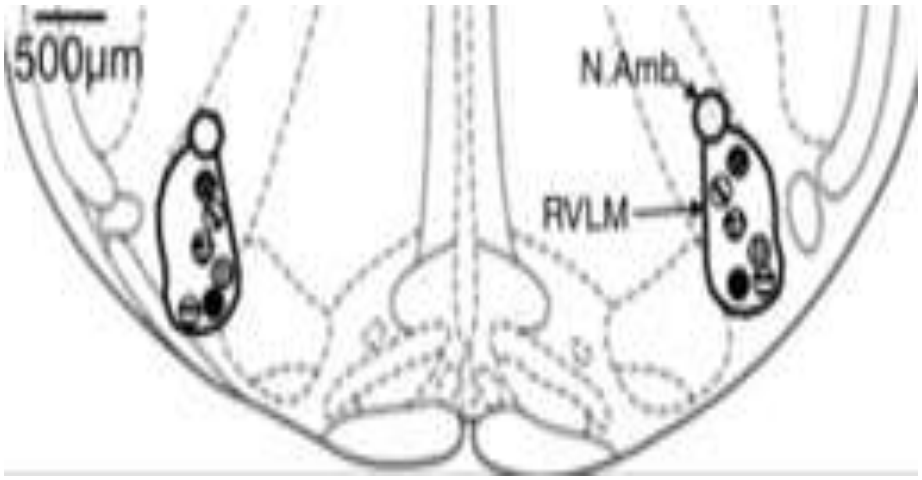

Figure 2-A coronal section through the rat brainstem from Paxinos and Watson [24], illustrating the position into the RVLM of the implanted stimulating electrode.

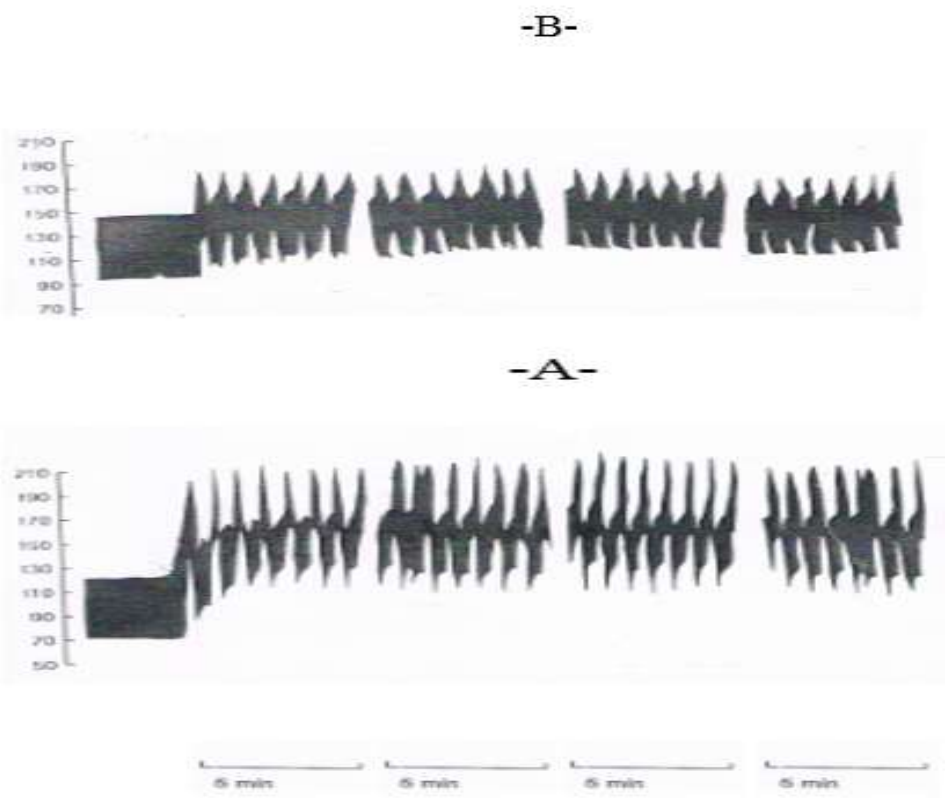

Figure 3-The basal SBP and the reproducibility of SBP responses, to a train of stimuli applied for 30min. Shows the responses during 5min immediately following stimulation (a), after 10min (b) after 20min (c), and after 30min (d) of RVLM-stimulation in urethane (A) and halothane (B) anaesthetized rats.

Results

Microscopic examine of the brain slices representing coronal sections through the brainstem regions of the urethane and halothane anaesthetized, RVLM sham - and RVLM-stimulated rats which used in these experiments, indicated that the stimulating electrode was located correctly inside the RVLM region for the experimental groups and outside it for the sham control. Diagrammatic representation in Figure-2 show the correct placement of stimulating electrode in both RVLM sham- and RVLM–stimulated animals.

Table-1 shows the values of basal SBP and HR, and the changes in BP and HR to the electrical RVLM sham- and RVLM-stimulated rats anesthetized with urethane or halothane (for 30 min with a 10 sec train of 1 msec square wave pulse duration at 40 Hz and 2 V every 30 sec), and control conscious rats received neither anesthesia nor stimulation. There were significant differences in the basal SBP (before stimulation) values of the rats anaesthetized with urethane (123±4 mmHg, 6 rats/group; $P<0.001$) compared to that of the control group which received neither anesthesia nor stimulation (140±6 mmHg, 6rats/group), or a group anaesthetized with halothane (143±6 mmHg, 6 rats/group). Electrical stimulation of the RVLM area, resulted in a significant reproducible elevation of SBP in both urethane and halothane anaesthetized groups. The pressor peak response occurred and decayed within 1-2 sec following the onset or termination of the stimulus. Urethane anaesthetized rats moreover showed a significant greater increase in BP in response to stimulation of the RVLM area, providing that there was an increase of $\Delta 94.0 \pm 4$mmHg in the urethane anaesthetized group, compared to $\Delta 38 \pm 4$mmHg with halothane. The magnitude of the maximum pressor response to RVLM stimulation was found to be constant and reproduced with the same intensity over the 30 min stimulation period Figure-3(A, B). There was no change in BP when the stimulation was made close but outside the RVLM area in both urethane and halothane anaesthetized rats Figure-1.

For studying the effects of urethane vs. halothane on plasma, NIL, ME, SON, or PVN level of AVP-LI, Figure-4(A)shows the effects of urethane and halothane on the plasma level of AVP-LI in 30min RVLM sham-stimulated urethane or halothane anaesthetized rats, compared to that of RVLM

stimulated urethane or halothane anaesthetized rats, and control rats neither anesthetized nor stimulated. There were significant differences between the effects of urethane and halothane on the plasma level of AVP. Plasma levels of AVP-LI of urethane sham-operated groups were significantly greater (40±5pg/ml; P<0.05) than either the control (11±3pg/ml) or halothane sham-operated groups (23±4pg/ml), whereas halothane produced no significant change in plasma levels of AVP, compared to the controls, indicated that urethane anesthesia by itself enhanced the release of AVP into the peripheral circulation, in contrast to halothane which luck of such influence. However, and as it shown in fig.4B, both of the anesthetics did not prevent the release of AVP into the circulation following to 30min stimulation of RVLM area (30±3 urethane vs. 29±3pg/ml halothane; p<0.05), indicated that stimulation of RVLM enhanced the hypothalamic release of AVP into the circulation regardless of the anesthetic being used.

Figure-5(A) shows the effects of urethane and halothane used to anesthetized the rats that either their RVLM stimulated or sham-stimulated for 30min, on the level of NIL AVP-LI, in comparison with that of control rats neither anesthetized nor stimulated. The level of NIL AVP-LI of urethane sham-operated rats were significantly lower (1495±20ng/gland; p<0.05) than either the control (1850±25ng/gland; p<0.05) or halothane sham-stimulated rats (1900±24ng/gland; p<0.05), which indicated that urethane by itself enhance the release of AVP of NIL into the circulation, in contrast to halothane which luck of such influence. The finding that both of the anesthetics did not prevent the release of AVP into the circulation following to 30min stimulation of RVLM area (1490±22ng/gland urethane vs. 1485±25ng/gland; Figure-5(B), also indicated that stimulation of RVLM enhanced the hypothalamic influence over the release of AVP of NIL into the circulation regardless of the anesthetic being used.

Figure-6(A) shows the effects of urethane and halothane on the ME level of AVP-LI in 30min RVLM sham-stimulated urethane or halothane anaesthetized rats, compared to that of RVLM stimulated urethane or halothane anesthetized rats, and control rats neither stimulated nor anesthetized. ME level of AVP-LI of urethane sham-operated rats were significantly lower (180±28ng/mg protein) than that of either the control (680±21ng/mg protein; p<0.05) or halothane sham-stimulated rats (785±70ng/mg protein; p<0.05), similarly indicated that urethane by itself enhance the release of AVP of ME into the circulation, in contrast to halothane which also luck of such influence. The finding that the reduction in the level of AVP-LI following 30min RVLM stimulation significant only in urethane anesthetized rats (320±17ng/mg protein; P<0.05) but not in halothane anesthetized rats (360±18ng/mg protein) Figure-6(B), indicated that the reduction in the level of AVP of ME is a product of urethane effects on hypothalamic-neurohypophysial system rather than a product of a direct influence of a subset of stimulated RVLM projection to the AVP-hypothalamic sites.

Figure-7(A) shows the effects of urethane and halothane on the SON level of AVP-LI in 30min RVLM sham-stimulated urethane or halothane anaesthetized rats, compared to that of RVLM stimulated urethane or halothane anesthetized rats, and unanesthetized unstimulated control rats. SON level of AVP-LI of urethane sham-operated rats were significantly lower (55±10ng/mg protein; p<0.05) than that of either the control (107±11ng/mg protein; p<0.05) or halothane sham-stimulated rats (110±14ng/mg protein; p<0.05), so indicated that urethane by itself enhance the release of AVP of SON, in contrast to halothane which also luck of such influence. On other hand, the finding that both of the anesthetics did not prevent the release of AVP from SON following to 30min stimulation of RVLM area (34±4ng/mg protein; halothane vs. 30±3ng/mg protein; urethane) Figure-7(B), also indicated that stimulation of RVLM enhanced the release of AVP of SON regardless of the anesthetics being used.

Figure-8(A) shows the effects of urethane and halothane on the PVN level of AVP-LI in 30min RVLM sham-stimulated urethane or halothane anaesthetized rats, compared to that of RVLM stimulated urethane or halothane anesthetized rats, and unanesthetized unstimulated control rats. PVN level of AVP-LI of urethane sham-operated rats did not affected by urethane anesthesia (890±30ng/mg protein) compare to that of either the control (990±50ng/mg protein) or halothane sham-stimulated rats (780±60ng/mg protein), indicated that urethane have no influence on the release of AVP from PVN, in contrast to halothane which by itself showed influence on the release of AVP from PVN. On other hand, the finding that both of the anesthetics did not prevent the release of AVP from SON following to 30min stimulation of RVLM area (600±30 ng/mg protein; urethane vs. 450±25ng/mg protein;

halothane) Figure-8(B), indicated a role for RVLM when stimulated, on the release of AVP of PVN regardless of the anesthetics being used.

Table 1-Basal BP and HR and changes in BP and HR at 6- and 1min before and 1-, 6-, 11-, 16-, 21-, and 26min after stimulation or sham stimulation of the RVLM of rats anesthetized with urethane and halothane, and a control rats received neither anesthesia nor stimulation. The comparison of the differences in the sequential values (before and after) of the same group (* P<0.05, ** P<0.01), or in the inter-groups values ($ P<0.05, $$ P<0.01), were done using respectively paired Student's t-test and unpaired Student's t-test. Values given as mean ±S.E.M. of 6 rats/group.

	Type of Stimulation	**Time before stimulation (min)**		**Time after stimulation (min)**					
		-6	**-1**	**+1**	**+6**	**+11**	**+16**	**+21**	**+26**
SBP mm Hg	RVLM-stim. Urethane	$ 123±4	$ 123±4	**$ +98±4Δ	**$ +98±4Δ	**$ +97±4Δ	**$ +92±4Δ	**$ +92±4Δ	**$ +91±4Δ
	halothane	143±6	143±6	*$ +40±6Δ	*$ +40±4Δ	*$ +40±4Δ	*$ +38±4Δ	*$ +38±4Δ	*$ +37±6Δ
	control	140±6	140±6	139±6	139±6	138±6	138±6	138±6	138±6
	Sham-stimul. urethane	$ 123±4	$ 123±4	$ 123±4	$ 123±4	$ 123±4	$ 121±4	$ 121±4	$ 120±4
	halothane	143±6	143±6	143±6	143±6	144±6	143±6	144±6	144±6
	control	140±6	140±6	139±6	139±6	138±6	138±6	138±6	138±6
HR b/min	RVLM-stim. Urethane	372±8	372±8	*$ +66±4Δ	*$ +66±4Δ	*$ +67±4Δ	*$ +67±4Δ	*$ +68±4Δ	*$ +68±4Δ
	Halothane	365±8	365±8	*$ +65±3Δ	*$ +66±3Δ	*$ +66±3Δ	*$ +67±3Δ	*$ +67±3Δ	*$ +67±3Δ
	control	368±9	368±9	368±9	367±9	367±9	368±9	368±9	369±9
	Sham-stimul. urethane	372±	372±8	372±8	372±8	371±8	272±8	374±8	374±8
	halothane	365±8	365±8	365±8	366±8	367±8	367±8	368±8	368±8
	control	368±9	368±9	368±9	367±9	367±9	368±9	368±9	369±9

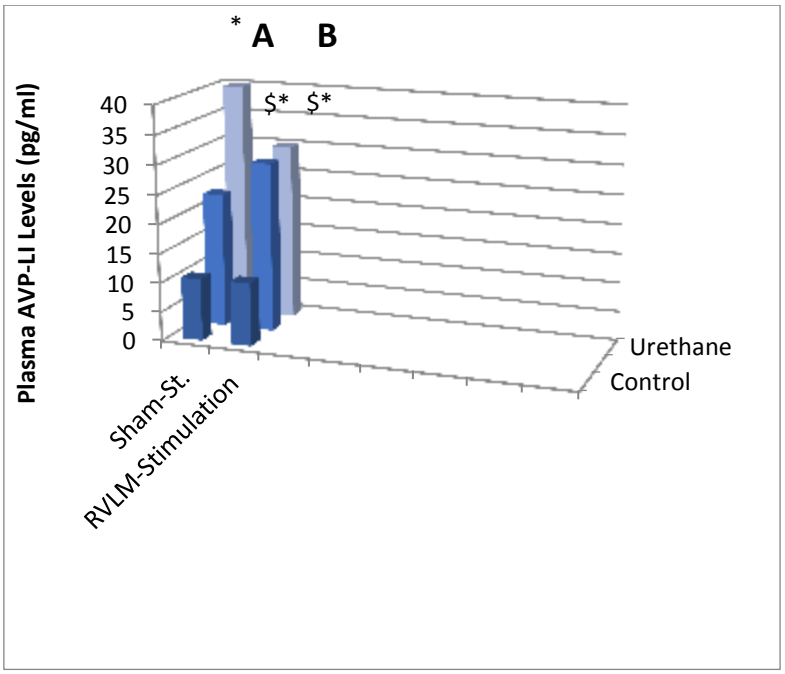

Figure 4-Plasma AVP levels following 30 min electrical stimulation of the RVLM region of the urethane (light blue columns A&B) or halothane anaesthetized rats (dark blue columns A&B), compared respectively either to that of urethane or halothane sham stimulated rats, or to that of control rats received no anesthesia or stimulation (black columns A&B). Stimulation parameters were 10 sec train of 1msec square wave duration at 40Hz and 2V every 30s for 30 min. *P<0.05 significant from rats received no anesthesia or stimulation, and §P<0.05 significant from halothane anaesthetized sham stimulated rats by using Fisher PLSD: one factor ANOVA. Results given as mean ±S.E.M. of 6 rats/group.

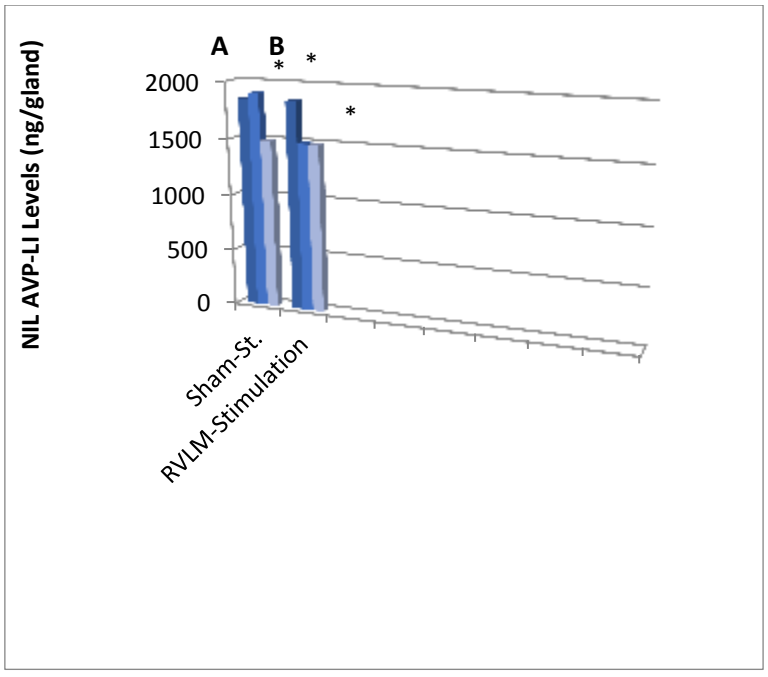

Figure 5-NIL AVP levels following 30 min electrical stimulation of the RVLM region of the urethane (light blue column) or halothane anaesthetized rats (dark blue column), compared respectively either to that of urethane or halothane sham stimulated rats, and to that of control rats received no anesthesia or stimulation (black columns). Stimulation parameters were 10 sec train of 1msec square wave duration at 40Hz and 2V every 30s for 30 min. *P<0.05 significant from rats received no anesthesia or stimulation, using Fisher PLSD: one factor ANOVA. Results given as mean ±S.E.M. of 6 rats/group.

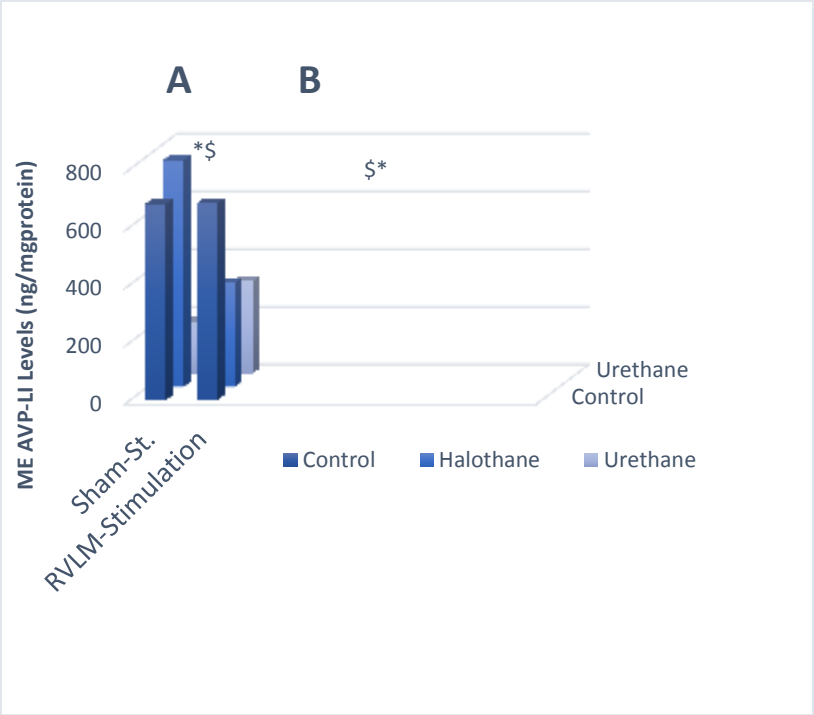

Figure 6-ME AVP levels following 30 min electrical stimulation of the RVLM region of the urethane (light blue column) or halothane anaesthetized rats (dark blue column), compared respectively either to that of urethane or halothane sham stimulated rats, and to that of control rats received no anesthesia or stimulation (black columns). Stimulation parameters were 10 sec train of 1msec square wave duration at 40Hz and 2V every 30s for 30 min. *P<0.05 significant from rats received no anesthesia or stimulation, using Fisher PLSD: one factor ANOVA. Results given as mean ±S.E.M. of 6 rats/group.

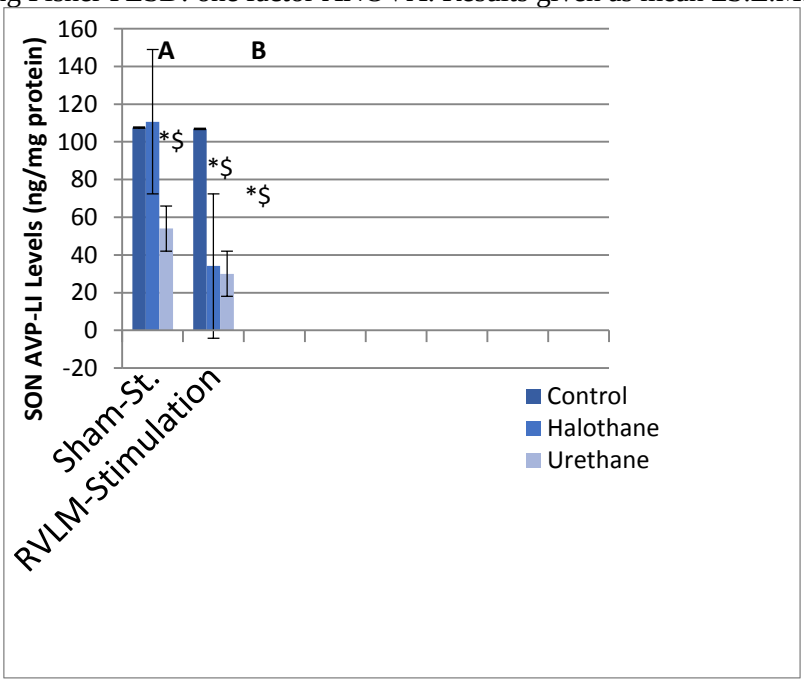

Figure 7- SON AVP levels following 30 min electrical stimulation of the RVLM region of the urethane (light blue column) or halothane anaesthetized rats (dark blue column), compared respectively either to that of urethane or halothane sham stimulated rats, and to that of control rats received no anesthesia or stimulation (black columns). Stimulation parameters were 10 sec train of 1msec square wave duration at 40Hz and 2V every 30s for 30 min. *P<0.05 significant from rats received no anesthesia or stimulation, and §P<0.05 significant from halothane anaesthetized sham

stimulated rats by using Fisher PLSD: one factor ANOVA. Results given as mean ±S.E.M. of 6 rats/group.

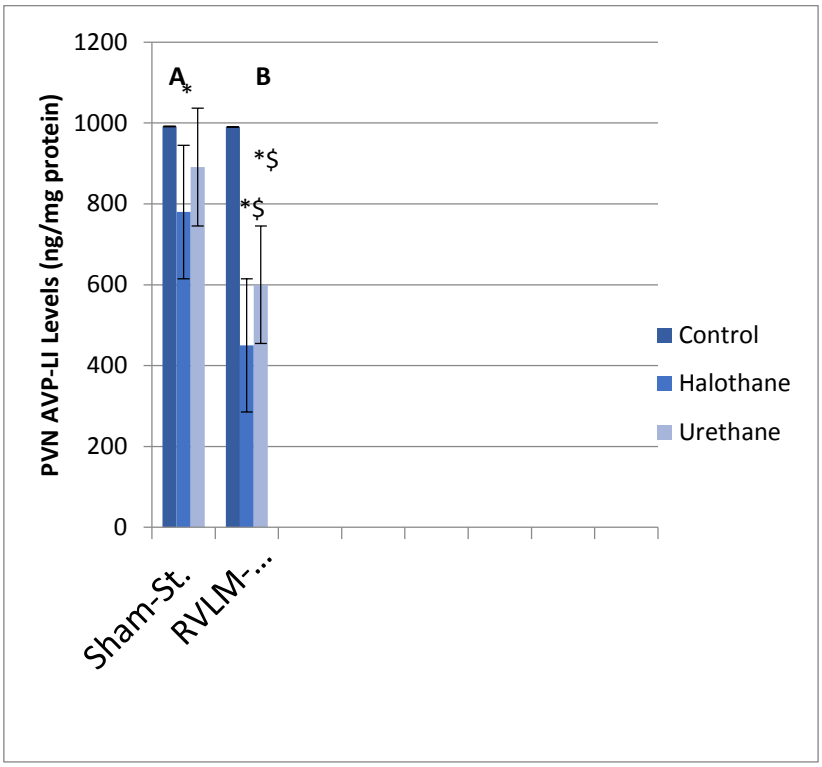

Figure 8-PVN AVP levels following 30 min electrical stimulation of the RVLM region of the urethane (light blue column) or halothane anaesthetized rats (dark blue column), compared respectively either to that of urethane or halothane sham stimulated rats, and to that of control rats received no anesthesia or stimulation (black columns). Stimulation parameters were 10 sec train of 1msec square wave duration at 40Hz and 2V every 30s for 30 min. *P<0.05 significant from rats received no anesthesia or stimulation, and §P<0.05 significant from halothane anaesthetized sham stimulated rats by using Fisher PLSD: one factor ANOVA. Results given as mean ±S.E.M. of 6 rats/group.

Discussion

This in vivo study illustrates the presence of differential effects between urethane and halothane on basal BP and the increase in BP to electrical stimulation of the RVLM region. Moreover, it is the first to demonstrate specific changes in AVP-LI at the level of the central limb of the hypothalamic-neurohypophysial-portal-system related to the stimulation of RVLM adrenergic neurons as well as to the effects of these two anesthetics.

Before discussing these results it is important to evaluate the protocol and procedure used in this study. Unilateral electrical stimulation for 30 min, using stimulating parameters of a 10 sec train of 1msec square wave pulse duration at 40 Hz and 2V every 30s, to the rostral VLM region both of urethane and halothane anaesthetized rats produced an increase in SBP. The preliminary study showed that the increase in SBP to the RVLM electrical stimulation was frequently dependent (data not shown), and a frequency producing a submaximal increase in SBP was selected and used in this study. This choice of frequency is consistent with the data published by Ross *et al*. [20] who showed the pressor response to appear at stimulus frequencies of 5 to 10Hz, when the current was held at 25μA, and to reach a maximum at frequencies between 150 and 300Hz. Moreover, these parameters resulted in a constant and reproducible pressor peak response with the same intensity over the 30 min stimulation both in urethane and halothane anaesthetized groups since the reductions of 12.2% (in urethane group) and 10% (in halothane group), in the increase of SBP during the period of RVLM stimulation, where not significant. Furthermore, the pressor peak responses occurred or decayed within 1-2sec following the onset or termination of the stimulus respectively, indicating that the procedure was without apparent damage to the stimulated neurons.

Related to resting BP, the basal SBP of halothane anesthetized rats was within the range of the resting SBP in conscious normotensive rats (non-anaesthetized non-stimulated controls), compared to a decrease in basal SBP showed in urethane anesthetized rats, implies that urethane in current study thus, produce a hypotension. The urethane hypotensive effect here is in good agreement with several lines of studies which have shown a reduction in the basal BP of the rats [30, 31]. There are many proposed mechanisms for urethane hypotensive effects. One of the mechanisms proposed at a cellular level, interference of urethane with Ca^{++} availability for contraction of vascular and tracheal smooth muscle [31,32], although Maggi et al. [31] believed that interference with transmembrane Ca^{++} fluxes would not seem to be a factor since this only occurs at concentrations higher than required for anesthesia. Another proposal mechanism is that urethane anesthesia inhibits cardiovascular responses that are mediated via peripheral and central α2–adrenoceptors [33]. There were no observations of cardiovascular responses when the stimulation was applied to areas outside the rostral VLM region. This indicated that the increase was related to activation of neurons within the discrete RVLM region. Although multiple types of neurons has been identified within the RVLM, at least one of these is the adrenergic neurons. A selective increase in hypothalamic AD levels to RVLM/C1 stimulation [19] and blood pressure rise to RVLM-microinjection of excitatory amino acids [20], which are considered to excite cell bodies or dendritic processes of neurons, but not axons of passages, together with the observation here of no cardiovascular responses to a stimulation applied to control area surrounding the RVLM/C1 region may all support the argument that the pressor response is due to activation of discrete adrenergic cells bodies, rather than stimulating fibers passing through the rostral VLM. In addition, it is unlikely that stimulation of the RVLM/C1 cell group will extend to the 5HT containing – B3 (pressor) cell group [34], since the coordinates where the stimulating electrode inserted are distinct [24], and the effects on BP observed in this study were similar in pressor peak magnitude, onset and decay to those seen in Ross *et al*. [20] and Goodchild *et al*. [35], following electrical or chemical stimulation of C1 adrenergic neurons. Comparable with the present results, the increase in BP following RVLM/C1 electrical stimulation has also been reported to occur in rats anaesthetized with urethane [36] and other anesthetics [19, 20].

In the present study, the increase in BP following RVLM stimulation, in urethane anaesthetized rats was unrestrained and 235% greater than that observed with halothane anesthesia, suggesting that the increase in BP during RVLM stimulation is anesthetic-dependent. The pressor response in halothane anaesthetized animals was in some way initially restrained and only improved after many attempts to change the position of the stimulating electrode within the RVLM area. This practical problem with halothane is consistent with the previous conclusion [37] that urethane might be the anesthetic of choice for its fairly good observation of both the cardiovascular reflexes and the activity of the autonomic nervous system controlling cardiovascular function. The reason of way urethane induced greater BP response following RVLM stimulation than that of halothane, might be clarified by the observations that the rostral VLM and caudal VLM neuronal activity are correlated to the effect of the two anesthetics on changes in BP. Previous experiments have reported that controlled hypotension increased rostral VLM and caudal VLM catecholaminergic neuronal activity [38], whereas controlled hypertension in the present study would therefore be expected to increase rostral VLM-adrenergic neuronal activity which is expressed as higher BP response to RVLM stimulation than that in the presence of halothane which shows no induced change in resting BP. Consistent with this is the suggestion that the magnitude of the hypotensive effect caused by placement of lesions in the rostral VLM is also anesthetic-dependent [39] since a more sever hypotension was observed in urethane anaesthetized animals following the lesions when compared with the use of other anesthetics.

The major measurements undertaken in the present study were those of AVP, in order to elucidate the changes of this peptide during stimulation of C1 adrenergic neurons. One problem however, is the previous evidence from several investigations which have reported that urethane and other anesthetics increase plasma AVP [20, 37, 40]. The present study showed that the increase in the plasma AVP-LI level after urethane was accompanied by a decrease in AVP-LI in the NIL, ME, and SON, but not the PVN. The results with urethane suggest that the decrease in AVP-LI levels in NIL, ME, and SON may be attributed to the observed increase in plasma AVP-LI. Furthermore, the absence of change in the PVN AVP-LI, suggests that the SON and PVN are differentially responsive to the effects of urethane. In contrast, the use of halothane resulted in no significant changes in AVP release or tissue levels except in the PVN again suggesting the SON and PVN are differentially responsive to the effect of

halothane. This implies that despite the difficulties with the use of halothane in studying cardiovascular responses this anesthetic is preferred in comparison with urethane to monitor AVP changes following RVLM stimulation, due to the apparent absence of an effect on AVP secretion. The results using urethane further raise the question whether the effects of this anesthetic on the AVP-LI levels at the sites of the hypothalamic-neurohypophysial-axis, are due to a central action or are reflex to the hypotensive effect. Although This is likely reflex to the peripheral hypotensive effects of urethane because; i) the urethane hypotensive action may be due to a peripheral vascular effect which is discussed above, and since the centrally controlled sympathetic tone was comparable after lesions of the rostral VLM using urethane and non-hypotensive anesthetics, as indicated by similar decrease in MAP after trimethaphan [39], ii) the firing rate of hypothalamic neurosecretory neurons was normal in urethane anaesthetized rats, in spite of the increased levels of plasma AVP [40], and iii) the finding of the current study that there was no change in AVP level of the PVN of the urethane-hypotensive rats. Therefore, one might assume that the urethane-induced release of AVP into the blood stream was in response to a peripheral reflex due to the hypotension rather than to a central action of urethane. Urethane anesthesia however, produces a functional blockade of pre- and post-synaptic α2-receptors peripherally and/or centrally [33] which raise the possibility of urethane acting centrally to prevent the inhibitory α2 mediated feedback on NA release from noradrenergic fibers terminating on the hypothalamic neurosecretory cells, which in turn activates α1–adrenoceptors to stimulate the release of AVP [41,42]. Further investigation to resolve this controversy may be essential.

The demonstration in this study that halothane has no effect on basal BP was not consistent with a previous study [43] where halothane was also hypotensive. However, the data concerning the effect of halothane on the plasma AVP level is in a good agreement with a previous data demonstrating that halothane and other anesthetic agents do not release AVP [5, 22, 44]. Moreover, the non- significant increase in plasma AVP-LI here agrees with Aziz and Forsling [45] who observed a slight increase in plasma AVP in halothane anaesthetized rats. The only effect of halothane observed was a small decrease in PVN-AVP-LI level suggesting that halothane, in contrast to urethane, may exert its effect centrally. Martin *et al*. [46] using brain synaptosomes demonstrated that halothane could result in acutely enhanced serotonergic neurotransmission which might effect AVP release. However it needs to be demonstrated that this was a concentration-dependent effect, and occurred at concentration relevant to anesthetic doses of halothane. Electrical stimulation of the RVLM region of the halothane anaesthetized rats, increased plasma AVP-LI levels; an effect not seen in halothane sham stimulated controls, and potentiated the decrease in PVN-AVP-LI by producing a further significant decrease in the PVN-AVP-LI level in comparison to that in halothane sham stimulated controls. Whereas further to increase plasma AVP-LI and decrease NIL, ME, and SON-AVP-LI levels, electrical stimulation of the RVLM region of the urethane anaesthetized rats, potentiate the decrease in SON-AVP-LI level in comparison to that in urethane sham stimulated controls, and more interestingly, caused a decrease in the PVN-AVP-LI level; an effect not observed in urethane sham-stimulated controls. Consistent with present data, is the previous finding that electrical stimulation of the RVLM region increased plasma AVP [20]. However, the finding of reduction in PVN-AVP level due to RVLM stimulation regardless of used anesthesia in present study, and that C1 stimulation may selectively increase extracellular hypothalamic AD, but not NA, DOPAC, and SHIAA, in view of previous study [19], suggest a role for stimulated RVLM/C1 adrenergic terminals in vicinity of the hypothalamic SON and PVN, in mediating the physiological stressors, providing that acute or strong physiological stressors including sever hypotension [21], increase markedly hypothalamic extracellular AD, or deplete markedly hypothalamic AD [47], and induce rapid release of PVN-parvocellular-AVP and corticotropin releasing hormone; CRH into the pituitary portal circulation [48]. AVP considers main determinant controlling ACTH secretion [2]. AVP stimulates pituitary ACTH secretion through interaction with receptors of the $V1_B$ subtype ($V1_BR$, V3R), located in the plasma membrane of the pituitary corticotroph, mainly by potentiating the stimulatory effects of CRH [49].

In summary, the results demonstrates the presence of differential effects of the anesthetics (urethane and halothane) on resting BP, the increase in BP following RVLM-stimulation and the hypothalamic AVP production and its release into the blood stream. Halothane produced no effect on resting BP, in contrast to a hypotensive effect produced by urethane which its effect on the pressor response to RVLM-stimulation was greater and unrestrained in comparison to that in halothane anaesthetized rats. However, to known whither the hypotension was due to a peripheral or central

mechanism needs further investigation. The anesthetics caused differential changes in AVP-LI at the level of the central limb of hypothalamo-neurohypophysial axis, which with urethane, in contrast to halothane, was more extensive and involves sites outside the blood brain barrier including NIL, ME, and blood plasma, suggesting different mechanisms of control of the release of AVP at those sites. RVLM stimulation however, abolished the regional specificity of the effects of the anesthetic on SON versus PVN-AVP levels, indicating that neither anesthetic prevented the central mechanisms involved in the response of AVP neurons to RVLM stimulation. In terms of the effects of the two anesthetics used in this study to monitor the regulation of AVP. In conclusion, halothane is preferred for examining the effects of RVLM-stimulation on AVP changes, whereas in term of their effects on the pressor response to stimulation of the RVLM area, urethane appears preferable

.

References

1. Stricker, E.M. and Sved, A.F. **2002.** Controls of vasopressin secretion and thirst: similarities and dissimilarities in signals. *Physiol. & Behavior*. **77**:731.
2. Antoni, F.A. **1993.** Vasopressinergic control of pituitary adrenocorticotropin secretion comes of age. *Front. Neuroendocrinol*.**14**: 76–122.
3. Coote, J.H. **2004**. *The hypothalamus and cardiovascular regulation In Neural mechanism of cardiovascular regulation* (eds. Dun N., Machado B. & Pilowsky P.). 117–146. Kluwer Academic Publishers, Boston, MA, USA.
4. Zhong, M.K., Duan, Y.C., Chen, A.D., Xu, B., Gao, X.Y., De, W. and Zhu, G.Q. **2008.** Paraventricular nucleus is involved in the central pathway of cardiac sympathetic afferent reflex in rats. *Exp. Physiol.* **93**(6):746-53.
5. Pyner, S. and Coote, J.H. **1999.** Identification of an efferent projection from the paraventricular nucleus of the hypothalamus terminating close to spinally projecting rostral ventrolateral medullary neurons. *Neuroscience*. **88**: 949–57.
6. Pyner, S. and Coote, J.H. **2000.** Identification of branching paraventricular neurons of the hypothalamus that project to the rostroventrolateral medulla and spinal cord. Neuroscience. **100**(3):549-56.
7. Chen, Q.H. and Toney, G.M. **2010**. *In vivo* discharge properties of hypothalamic paraventricular nucleus neurons with axonal projections to the rostral ventrolateral medulla. *J Neurophysiol*.**103**: 4–15.
8. Kc, P., Balan, K.V., Tjoe, S.S., Martin, R.J., LaManna, J.C., Haxhiu, M.A. and Dick, T.E. **2010.** Increased vasopressin transmission from the paraventricular nucleus to the rostral medulla augments cardiorespiratory outflow in chronic intermittent hypoxia-conditioned rats. *J Physiol*. **588**:725–740.
9. Mack, S.O., Kc, P., Wu, M., Coleman, B.R., Tolentino-Silva, F.P., and Haxhiu, M.A. **2002.** Paraventricular oxytocin neurons are involved in neural modulation of breathing. *J Appl Physiol.* **92**(2): 826-34.
10. Kumagai, H., Oshima, N., Matsuura, T., Iigaya, K., Imai, M., Onimaru, H., Sakata, K., Osaka, M., Onami, T., Takimoto, C., Kamayachi, T., Itoh, H. and Saruta, T. **2012.** Importance of rostral ventrolateral medulla neurons in determining efferent sympathetic nerve activity and blood pressure. *Hypertens. Res*. **35**: 132–141.
11. Kumagai, H., Oshima, N., Matsuura, T., Imai, M., Iigaya, K., Onimaru, H., Kawai, A., Sakata. K., Onami, T., Takimoto, C., Kamayachi, T., Osaka, M., Hayashi, K., Itoh, H. and Saruta, T. **2007.** Whole-cell patch-clamp and optical imaging of neurons in the RVLM, the CVLM, and the caudal end of VLM In: Central Mechanisms of Cardiovascular Regulation, ed. Kubo T, editor; & Kuwaki T, editor. pp. 83–106. Transworld Research Network, Kerala.
12. Feng, K. and Guo, XQ. **1997**. Effect of vasopressin in the rostral ventrolateral medulla on pressor response induced by dPAG stimulation. *Sheng Li Xue Bao.* **49**(5): 491-6.
13. Zhao, Y.H., Shen, X.H. and Guo, X.Q. **2000**. AVP-ergic mechanism in the rostral ventrolateral medulla:a possible role in stress-induced hyperviscosity. *Sheng Li Xue Bao*. **52**(3): 255-8.
14. Philip, R.G., Stephen, J.L., Louise E. H., Julian, F.R.P., and Anne-Marie O'Carroll. **2017**. Vasopressin V1a receptors mediate the hypertensive effects of [Pyr^{1}] apelin-13 in the rat rostral ventrolateral medulla. *J Physiol*. **595**(11): 3303–3318

15. Cheng, M.T., Chuang, C.W., Lin, J.T. and Hwang, J.C. **2004.** Cardiopulmonary response to vasopressin-induced activation on V1A receptors in the lateral ventro-lateral medulla in the rat. *Chin J Physiol*. **47**(1): 31-42.
16. Chuang, C.W., Cheng, M.T., Yang, S.J. and Hwang, J.C. **2005.** Activation of ventrolateral medulla neurons by arginine vasopressin via V1A receptors produces inhibition on respiratory-related hypoglossal nerve discharge in the rat. *Chin J Physiol.* **48**(3): 144-54.
17. Prabha, K., Balan, K.V., Martin, R.J., Lamanna. J.C., Haxhiu, M.A. and Dick, T.E. **2011**. Chronic intermittent hypoxia-induced augmented cardiorespiratory outflow mediated by vasopressin-V_1A receptor signaling in the medulla. *Adv Exp Med Biol.* **701**:319-25.
18. Tucker, D., Saper, CB., Ruggiero, DA., *et al,*. **1987**. Organization of central adrenergic pathway: I: Relationships of ventrolateral medullary projections to the hypothalamus and spinal cord. *J. Comp. Neurol*. **259**: 591-603.
19. Routledge, C. and Marsden, C.A. **1987.** Electrical stimulation of the C1 region of the rostral ventrolateral medulla of the rat increases mean arterial pressure and adrenaline release in the posterior hypothalamus. *Neurosci*. **20**: 457.
20. Ross, CA, Ruggiero, DA, Park, DH, Joh, T.H., Sved, A.F., Fernanzez-Pardal, J., Saavedra, J.M. and Reis, D.J. **1984**. Tonic vasomotor control by the rostral ventrolateral medulla: effect of electrical or chemical stimulation of the area containing C1 adrenaline neurons on arterial blood pressure, heart rate and plasma catechol amines and vasopressin. *J. Neurosci*. **4**: 474-94.
21. Philippu, A., Dietl, H. and Sinha, J.N **1979.** In vivo release of endogenous catecholamine in the hypothalamus. *Naunyn-Schmiedeberg's Arch. Pharmacol*. **308**: 137-142.
22. Philbin, D.M. and Coggins, C.H. **1987.** Plasma antidiuretic hormone levels in cardiac surgical patients during morphine and halothane anesthesia. *Anaesth.***49**: 95-8.
23. Engler, D., Pham, T., Fullerton, M.J., et al. **1989**. Studies of the secretion of corticotropin-releasing factor and arginine vasopressin into the hypophysial-portal circulation of the conscious sheep. I. Effect of an audiovisual stimulus and insulin-induced hypoglycemia. *Neuroendocrinol.* **49**: 367–381.
24. Paxinos, G. and Watson, C. **1986.** *The rat brain in stereotaxic coordinate*. 2nd ed., Academic Press. Australia.
25. Rashid, I.H. **2017**. Role of RVLM/C1-α2-Adrenoceptors in Mediating the Effects of Clonidine on Blood Pressure and Heart Rate is Anesthetic Dependent. Accepted for publication.
26. Palkovits, M. and Brownstein, M. **1988**. *Maps and guide to microdissection of the rat brain*. Elesvier, New York, Amsterdam. London,
27. Lowry, O.H., Rosenberg, N.J., Lewis Farr, A., *et al*. **1951**. Protein measurement with the folin phenol reagent. *J. Biol. Chem*. **193**: 265.
28. 35- Rooke, P. and Baylis, P.H. **1983**. A new sensitive radioimmunoassay for plasma arginine vasopressin. *J. Immunoassay*. **3**:115-131.
29. Jenkins, J.S., Ang. V.T., Hawthorn, J. and Rossor, M. **1983**. *Quantitative distribution of neurohypophysial hormones in human brain and spinal cord. In*: The Neurohypophysis. Progress in brain Research. (Eds) B.A. Gross and G. Leng. 60, pp.123-28. Elsevier. Amsterdam.
30. Sapru, H. N. and Krieger, A.J. **1979**. Cardiovascular and respiratory effect of some anesthetics in the decerebrate rat. *Eur. J. Pharmacol*. 53: 151-58.
31. Maggi, C.A., Manzini, S., Parlani, M. and Meli, A. **1984**. An analysis of the effects of urethane on cardiovascular responsiveness to catechol amines in terms of its interference with Ca^{++}. *Experientia*. **40**: 52–59.
32. Bennett, T. and Gardiner, S.M. **1985**. Hypotension following antagonism of the cardiovascular actions of vasopressin in urethane-anesthetized Long Evan, Wistar and Sprague-Dawley rats. *J. Physiol*. **366**: 51p
33. Armstrong, J.M., Lefevre-Borg, F., Scatton, B. and Cavero, I. **1982**. Urethane inhibits cardiovascular responses mediated by the stimulation of α_2-adrenoreceptors. *J. Pharmac. exp. Ther*. **223**: 524–535.
34. Chalmers, J.P., Minson, J.B. and Choy,V. **1984**. Bulbospinal serotonin pressor pathways and hypotensive action of methyldopa in the rat. *Hypertens.* **6**(II): 16-21.
35. Goodchild, A.K., Moon, E.A., Dampney, R.A.L. and Howe, P.R.C. **1984**. Evidence that neurons in the rostral ventrolateral medulla have a vasopressor function. *Neurosci. Lett*. **45**: 267-72.

36. Batchelard, H., Rivest, R. and Marsden, C.A. **1991**. Posterior hypothalamic receptors involved in the cardiovascular changes elicited by electrical stimulation of the rostral ventrolateral medulla. *Neuropharmacol*.**30**: 753-762.
37. Maggi, C. A. and Meli, A. **1986.** Suitability of urethane anesthesia for physiopharmacological investigations in various system. Pt 2: Cardiovascular system. *Experientia*. **42**: 292-97.
38. Milne, B., Quintin, L. and Gillon, J.Y. **1990**. Change in catecholamine metabolism in the crostral ventrolateral medulla following halothane and nitroprusside-induced hypotension; an in vivo electrochemical study. *Brain Res*. **518**: 143-48.
39. Cochrane, K.L., Buchholz, R.A., Hubbard, J.W., Keeton, T.K. and Nathan, M.A. **1988.** Hypotensive effects of lesions of the rostral ventrolateral medulla in rats are anaesthetic dependent. *J. Auto. Nerv. Syst*. **22**: 181-87.
40. Dyball, R.E.J. and McPhail, C.I. **1974**. Unit activity in the supraoptic and paraventricular nuclei. Effect of anesthetics. *Brain Res*. **67**: 43-50.
41. Day, T.A., Ferguson, A.V. and Renaud, L.P. **1984.** Facilitatory influence of noradrenergic afferents on the excitability of rat paraventricular nucleus neurosecretory cells. *J.Physiolo*.**355**: 237.
42. Day, T.A., Randle, J.C.R. and Renaud, L.P. **1985**. Opposing α- and β-adrenergic mechanisms mediated dose-dependent actions of noradrenaline on supraoptic vasopressin neurons in vivo *Brain Res*. **358**: 171-79.
43. Chen, B.B., Nyhan, D.P., Fehr, D.M., Goll, H.M. and Murray, P.A. **1990**. Halothane anesthesia causes active flow-independent pulmonary vasoconstriction. *Am. J. Physiol*. **259**: H74-83.
44. Husain, M.K., Manger, W.M., Rock, T.W., Weiss, R.J. and Frantz, A.G. **1979.** Vasopressin release due to manual restraint in the rat: role of body compression with other stressful stimuli. *Endocrinology*. **104**; 641-44.
45. Aziz, L.A. and Forsling, M.L. **1979.** Anesthesia and vasopressin release in rats. *J. Endocrinology*. **81**: 123.
46. Martin, D.C., Adams, R.J. and Introna, R.P.S. **1990.** Halothane inhibits 5-hydroxy-tryptamine uptake by synaptosomes from rat brain. *Neuropharmacol*. **29**: 9-16.
47. Roth, K.A., Mefford, I.M. and Barchas, J.D. **1982.** Epinephrine, norepinephrine, dopamine, and serotonine: differential effects of acute and chronic stress. *Brain Res*. **239**: 417-24.
48. Berkenbosch, F., de Goeij, D.C. and Tilders, F.J. **1989.** Hypoglycemia enhances turnover of corticotropin-releasing factor and of vasopressin in the zona externa of the rat median eminence. *Endocrinology*. **12**5: 28–34.
49. Volpi, S., Rabadan-Diehl, C. and Aguilera, G. **2004** Vasopressinergic regulation of the hypothalamic pituitary adrenal axis and stress adaptation. *Stress.* **7**(2): 75-83.