Role of the Anesthetic Determine the Clonidine Effects Mediated by RVLM/-α2-Adrenoceptors on Blood Pressure and Heart Rate in Rats

دور نوع المخدر في تاثيرات الكلوندين المعتمدة على مستقبلات ألفا -2- الادرينيرجيكية/ منطقة RVLM على ضغط الدم ودقات القلب في الجرذان

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Abstract

To examine the hypothesis of a role for α2-adrenoceptors in mediating the mechanism of urethane hypotensive effect whether it's peripheral or central, Wistar rats were anesthetized with urethane or (for comparison) with halothane, to study the influence of urethane that govern the mechanism of central and peripheral α2-adrenoceptors action, on basal BP & HR, and the rise in blood pressure (BP) to the stimulation of caudal pressor area (CPA), when these receptors were either centrally activated by bilateral rostral ventrolateral medulla (RVLM) microinjection of clonidine (30nM), and blockade with any of the clonidine antagonists, yohimbine (500pmol/50nl), and idazoxane (270nM) or yohimbine+idazoxane, or when peripherally activated (of urethane anesthetized rats) by i.v. clonidine (100nmol/kg), which also blockade with idazoxane or yohimbine+idazoxane. The results indicated presence of no anesthetic differences in a partial involvement of α2-receptors-RVLM, vs. a complete involvement of I(1)-imidazole receptors in mediating the hypotensive effects of clonidine. It also indicates α2-/I(1)-receptors synergism in raising the urethane lowering of baseline of SBP to the levels of control or halothane group. In conclusion, the result suggests involvement both of the central and the peripheral α2-adrenoceptors in mediating urethane hypotensive effects.

Key words: Urethane, halothane, clonidine, yohimbine, idazoxane, CPA, RVLM.

الملخص

لاختبار فرضية دور مستقبلات α2-الادرينيرجيكية في توسط آلية تأثير مادة التخدير يوريثان (urethane) الخافظة لضغط الدم، هل هو عن طريق محيط الجسم أم الجهاز العصبي المركزي، تم تخدير جرذان ويستار (Wistar) بمخدر يوريثان وللمقارنة بمخدر هالوثان (halothane) لدراسة الية تأثير اليوريثان التي تحكم عمل مستقبلات α2 في محيط ومركز الجسم (الدماغ) على كل من ضغط الدم ومعدل ضربات القلب الاساسية (basal)، وعلى ارتفاع ضغط الدم نتيجة تحفيز منطقة CPA, وذلك اولا عند تحفيز هذه المستقبلات مركزيا عن طريق حقن30 نانومول من الكلوندين (clonidine) في منطقة RVLM على جانبي الدماغ, ومنع مفعول الكلوندين بواسطة 500 بايكومول من مضاده النوعي على مستقبلات α2 اليوهمبين (yohimbine), او بواسطة 270 نانومول من مضاده النوعي على مستقبلات الاميدوزول I-1 الايدازوكسان (idazoxane), او بواسطة حقن المضادين معا (yohimbine+idazoxane), او ثانيا عند تنشيطها محيطيا عن طريق حقن 100 نانومول / كغم من الكلوندين في اوردة جرذان ويستر قبل تخديرها باليورثين, ومنع تاثير الكلوندين بحقن منطقة RVLM بمضاد الايدازوكسان بعد التخدير او بمادتي يوهيمبين + إدازوكسان. أشارت النتائج إلى عدم وجود اختلافات بين تاثير مواد التخدير المستخدمة على المشاركة الجزئية لمستقبلات α2، مقابل المشاركة الكاملة لمستقبلات I-1 -imidazole في توسطها تأثيرات الكلوندين الخافضة للضغط. كما اشارت النتائج ايضا الى وجود دور تازري في منع تاثير اليوريثان الخافض لضغط الدم الاساسي ورفعه إلى مستويات ضغط الدم في كل من مجموعتي السيطرة و مجموعة الهالوثين. يستنتج من نتائج الدراسة اشتراك كل من مستقبلات α2 المركزية والمحيطية في توسط تاثير اليوريثان الخافض لضغط الدم.

الكلمات الدالة: يوريثان، الكلوندين،CPA, RVLM، اليوهمبين ،الهالوثان، لايدازوكسان

Introduction

The optical imaging demonstrated that a medullary longitudinal rostrocaudal column, coincide the rostroventrolateral reticular medulla (RVLM) and the caudal end of ventrolateral medulla, acts as a sympathetic center [1], involved in the generating of resting sympathoexcitatory tone related to arterial pressure control, as well as a vital site of sympathoinhibitory actions of centrally acting antihypertensive agents [2,3]. The RVLM premotor neurons project directly and transmitted activity to sympathetic preganglionic neurons, the intermediolateral cell column, at each level of the spinal cord, where peripheral sympathetic nerves to the heart, arterioles and kidneys, acts to increase BP and involves in the maintenance of resting sympathetic vasomotor tone. An important part of sympathetic premotor neuron tonic activity of the RVLM, is driven by neurons located in a third region of the ventrolateral medulla govern caudal pressor area (CPA). The CPA is a pressor region located at the extreme caudal part of the ventrolateral medulla that appears to have an important role in controlling the activity of RVLM neurons [3]. CPA neurons activation elicit increases in RVLM neuronal discharge, vasoconstrictor sympathetic tone, and arterial pressure [4]. The pathway from CPA to RVLM involves an obligatory glutamatergic activation of sympathoexcitatory neurons in the vicinity of the

caudal ventrolateral medulla [5]. The micromapping study pinpointed the precise location of CPA neurons in a restricted region lateral to the caudal end of the lateral reticular nucleus and ventromedial to the medullary dorsal horn near the level of the pyramidal decussation. The intra-RVLM acting antihypertensive clonidine-like drugs lowers sympathetic tone first thought by activating alpha 2-adrenoceptors. According to the evolving later of the imidazoline hypothesis, clonidine binds not only to alpha 2-adrenergic receptors, but also to non-adrenergic specific sites, assuming the existence of a new group of receptors, the imidazole receptors, and attributes the sympathoinhibition to activation of I1-imidazoline receptor subtype [2]. Although the contribution of the two receptors in mediating the mechanism of action of the central antihypertensive effects, yet unresolved debate, the Peripheral Clonidine's blood pressure lowering effect is proposed to be mediated by both an immediate decrease in vascular resistance and a prolonged decrease in cardiac output, although of the believe that clonidine lowers central systolic blood pressure (SBP) more than peripheral SBP [6].

Numerous studies have concluded that urethane anesthesia provided a suitable condition for acute studies concerned with the physiopharmacology of various reflex responses at the cardiovascular level [7,8]. Depending upon dose, route of administration, and species, urethane caused a differential effects on BP and HR [7,8]. Although, in comparison with that of un-anaesthetized animals, urethane (1.5g/kg. i.p.) has reported to lower significantly resting HR and SBP [7], and produces a variety of metabolic and endocrine effects [9,10,11], in contrast to several anesthetic drugs like pentobarbital, halothane and ether which were shown not to cause such effects [12,13], with exception of a small increase was documented with halothane by others [14]. Furthermore, urethane anesthesia inhibits cardiovascular responses that are mediated by peripheral and central α2-adrenoceptors, providing that the selective α2–adrenoceptor agonist, oxymetazoline did not produce bradycardia in urethane anaesthetized animals [15]. Although, it is not clear yet whether urethane exert its hypotensive effects centrally and/or peripherally.

The experiment paradigm of this study therefore, is to study the impact of urethane on basal BP and HR, and the increase in BP to 5min CPA-stimulation in normotensive Wister rats, before and after subjecting of these hemodynamic parameters to the peripheral or intra-RVLM lowering effects of clonidine. The protocol was designed to study the antagonistic effect of bilaterally intra-RVLM injected α2-/I (1)-imidazole antagonists, prior to injection of clonidine, on the basal BP&HR, and on the increase in BP&HR to unilateral CPA electrical stimulation, in urethane anesthetized rats, and compared the results with that of halothane anesthetized and control rats. The effects of clonidine in other experiments may antagonized by prior administration peripherally (i.v.) or centrally (intra-RVLM) with antagonists either of clonidine affinity to α2-adrenoceptors, like yohimbine, or of clonidine affinity to I (1)-imidazole receptors, like idazoxane, or the both. Obtained data will compare with that obtained from applying similar protocol on halothane anesthetized rats or un-anesthetized sham CPA-stimulated rats, in order to determine whether urethane exert its hypotensive effects centrally and/or peripherally, as well as to interpret the mechanisms by which urethane may exert its hypotensive effect to avoid involvement of urethane with the effects of RVLM stimulation.

Materials and Methods

The experiments were performed on mature male Wister rats (250-300g weight) maintained under 12h light: 12h dark photoperiod with food and water available ad libitum. In one experiment, sought to investigate the hypotensive effect of urethane and the influence of urethane on the mechanism of hypotensive effects of clonidine. The rats under any of the anesthetic thus, were divided into five treated groups each of 6; a) two control groups received either vehicle or clonidine, b) three groups received either of clonidine antagonists, the selective α2-adrenoceptors, yohimbine, the selective I(1)-imidazol, idazoxane, or yohimbine+idazoxane, 2-3minutes prior to injection of clonidine. The vehicle or clonidine in these groups injected after and 15min before 5min stimulation of CPA region. The data of each treated group of the urethane anesthetized rats was compared with that received a vehicle or with that received same treatment but anesthetized with halothane. 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% O_2 and 50% NO_2, reduced to 2% during the surgical procedure, and to 1% after surgery until the end of the experiment.

In another experimental protocol designed to investigate the possible role for peripheral α2-adrenoceptors in mediating the hypotensive effect of urethane, the central manipulation of peripheral clonidine hemodynamic effects, was studied in rats divided into three groups each of 6; a) two control groups; a conscious normotensive group of rats to record the basal BP and HR, and urethane anesthetized group of rats received a vehicles i.v. and then intra-RVLM, b) a group of rats anesthetized with urethane (i.p), and then administered with clonidine (i.v.) followed by bilaterally injection into

the RVLM of either clonidine antagonists, idazoxane or idazoxane+yohimbine. The effects of clonidine on the resting BP&HR, were compared with that blocked by the antagonists or with the control conscious normotensive rats.

For blood pressure measurements following anesthesia, a skin incision over the thigh exposing of blood vessels, a polyethylene catheters (PE100) either through a 1ml syringe containing heparinized (30units/ml) saline (w/v) connecting to 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. Body temperature was maintained between 36 & 37C^0 by leaving the animal under an appropriate heat source. Systolic and diastolic BP & HR were monitored continuously throughout the experiment, by connecting the catheter fixed to the femoral artery to a Bell and Howell 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). All drugs were freshly prepared in normal saline (w/v) or 0.4M acetic acid (for idazoxane) which was used for control injection. Drugs and their vehicles were injected in a volume 0.2µl, delivered by hand over a period of 1.5-2min.

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 3.3mm above the intraaural line. Exposed and cleaned skull and cranium over the area of the medulla oblongata, then drilled to make a burr hole without piercing the Dura. A concentric needle electro-stimulator SNE100 (Clarke Electrochemical Equipment, 100µm.o.d) connected to the stimulating apparatus (Farnll Physiological Stimulator, England) set to deliver current at 10sec trains with a pulse duration of 1msec at 40Hz and 2volts every 30sec through the electrode, was inserted into the CPA region, using the following co-ordinates; 1.0 mm caudal to the calamus scriptorius, 2.0 mm lateral to the midline, and 1.7 mm ventral from the dorsal surface of the medulla (16), whereas the injection was done through a 20mm length of 31gauge stainless steel guide cannula, stereotaxic ally mounted bilaterally into the RVLM, in a position dorsal to the targeted area of the CPA 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 [17]. The same procedure of animal preparation, and cannulation to that described above.

The most active sites of the CPA area was explored by finding the area that produced the maximum increase in BP using single 10sec train of 1msec square wave pulse duration at 40Hz and 2V, which is the site where the electrode stayed in position throughout the experiments. After implantation the rats were left up to one hour for the BP to maintain a stable base line before further experimentation. Thereafter, SBP peak variations to resting and electrical stimulation of the CPA region were calculated at 5min intervals to compare the BP magnitudes and reproducibility during the experiment fig.(1). There was no change in BP following stimulation when the electrode was in an area 1mm dorsal, rostral, and lateral to the CPA area (co-ordinates described above; 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 15sec in order to mark the site of stimulation, or methylene blue to mark the site of injection. Then the stimulating electrode and the implanted cannula was removed and the rat immediately decapitated, and brain removed for dissection of brainstem [18]. Sections of the position of the stimulating electrode or implanted cannula was verified under a light microscope figure (3).

Data Analysis: Results were expressed as mean ± S.E.M. and n. is the number of the animals. Student's paired t-test were used for statistical analysis of the effects of the drugs on the basal and the CPA-stimulation pressor response in the same animal by measuring and comparing the before treatment mean values (control values) both of the before basal SBP and HR, and the increase in SBP and HR (Δ) during CPA-stimulation, with that of post-drug or -vehicle treatment (experimental) values. Student's unpaired t-test were used to assess the inter groups comparisons of the values. There were no significant differences in pre-vehicle control values for each group of animals, and so the values on the figures are the mean values of all the groups. Differences were considered significant at $p \leq 0.05$.

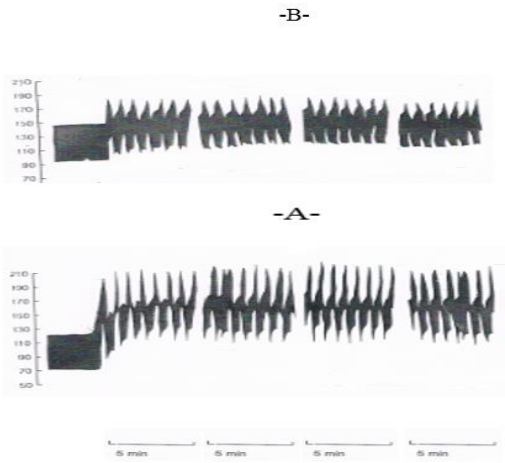


Fig. (1):Traces showing the basal SBP and the reproducibility of SBP responses to CPA-stimulation (with train of stimuli applied for 30min) during first 5min stimulation (a), after 10min (b), after 20min (d), and after 30min (d) in urethane (A) and halothane (B) anaesthetized rats.

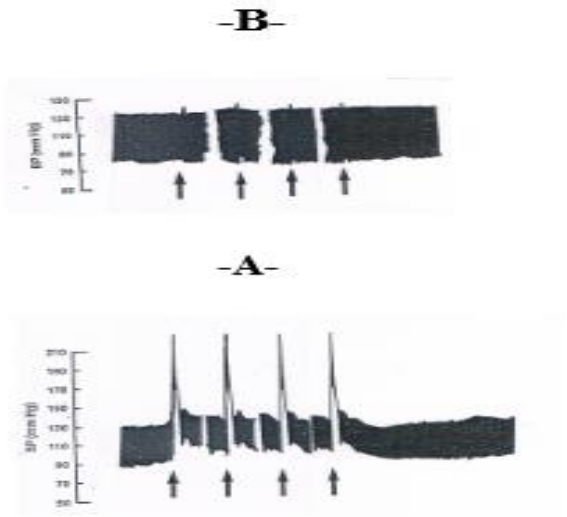


Fig. (2):Traces showing the SBP responses during a single electrical stimulation (↑) to the CPA region (A), or to the control areas outside the CPA region (B), with a 10sec trains of 1msec square wave pulse duration at 40Hz and 2volts.

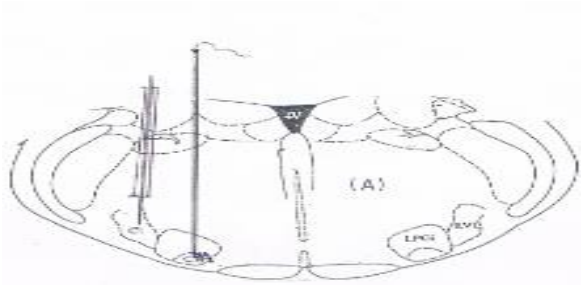


Fig. (3):Diagrammatic representation of a coronal section through the rat brainstem from Paxinos & Watson (1986), illustrating the position of the guide cannulae implanted into the RVLM region, and the stimulating electrode into the CPA area.

Results

Microscopical examination of the brain slices representing coronal sections through the brainstem RVLM and CPA regions of the normotensive urethane and halothane anaesthetized rats used in present experiments indicated that most of the injected cannulas were located in the correct targeted sites. The results from incorrect implantation 10% were not

used in the data analysis. Diagrammatic representation in fig. (3) shows the correct placement of injecting cannula or stimulating electrode in their region.

Electrical stimulation of the CPA area for 5min with a 10sec trains of 1msec square wave pulse duration at 40Hz and 2V every 30sec, resulted in a significant elevation of SBP in both urethane and halothane anaesthetized groups. The magnitude of the maximum pressor response to CPA-stimulation was found to be constant and reproduced (+94±5 mmHg in urethane vs. +39±5mmHg in halothane/96 rats), and with the same intensity over the 30min stimulation period, as well as over a period of about 2hr (the time of the experiment), using consecutive stimulation of 5min), indicating that this procedure caused no apparent damage to the CPA area neurons fig. (1). The presser peak response occurred and decayed within 1-2sec following the onset or termination of the stimulus.

Blood Pressure and Heart Rate Effects of Anesthetics in Wister Rats

Figure (4) shows the effects on basal SPB and the increase in BP to 5min electrical stimulation of CPA region, of urethane or halothane anesthesia in Wistar rats in comparison with that of rats received no anesthesia or stimulation (Δ). The Basal SBP of conscious rats (6/group) was 140±6mmHg, and did not change significantly in rats under halothane anesthesia (143±6mmHg, 6rats/group), but it was significantly lower (123±4mmHg, 6rats/group; $P<0.01$) in rats under urethane anesthesia. The responses in SBP to 5min electrical stimulation of unilateral CPA region in control rats under influence of halothane anesthesia (Δ+39±3.4mmHg) was significantly ($P<0.001$) augmented in control rats under the influence of urethane anesthesia (Δ+94±5.1mmHg). However, no change was made in BP when the stimulation close but outside the CPA area in both urethane and halothane anaesthetized rats. Figure (5) however, shows that basal HR in both anesthetized rat groups did not differ significantly (372±9b/m urethane vs 365±8b/m halothane) from the values of Conscious (368±9 control) rats. It also shows that the effects of the urethane and halothane on the responses in HR to CPA-stimulation generally, revealed no significant differences between the anesthetics (+67±4b/m vs. +66±4b/m respectively), although in few animals the increase in the HR was preceded by bradycardia. The HR was either gradually returned to pre-stimulation levels or was replaced by bradycardia, following the termination of the stimulus. There was also no significant change in basal SBP or HR, or the responses in SBP or HR to CPA-stimulation, after intra-RVLM administration of the vehicle in any of the experiments.

Anesthetics Influence on Clonidine Antihypertensive Effect

Figure (6A2&B2) shows the influence on the hypotensive effects of clonidine (30nM) bilaterally microinjected into RVLM, of urethane that used to anesthetize rats, in comparison with rats anesthetized with halothane or received no anesthesia or stimulation. Microinjection of clonidine produced hypotension with a differential decreases in the baseline of SBP under influence of urethane (75±3mmHg), in comparison with that of halothane (91±4mmHg). But, the magnitude of the hypotension was almost equal in both groups of anesthetic, since the reduction was -38% in urethane vs. -34% in halothane. Figure (7-A2&B2) shows that clonidine was significantly reduce the increases in SBP to CPA-stimulation, both in urethane and halothane groups, but the reduction in urethane was augmented (-58%) in comparison with -36% of halothane, indicated that the significant higher increase in basal SBP to CPA-stimulation in urethane than in halothane, may explained by the exclusive hypotensive effect of urethane but not halothane, and also by the significant lowering effect of urethane than halothane, on the baseline of SBP, and on the increase in BP to CPA-stimulation following injection of clonidine.

As shown in figure (8-A2&B2), both urethane and halothane anesthetized groups were showed a significant ($P<0.001$) decreases in the basal HR following bilateral intra-RVLM injection of clonidine, by -44% & -47% respectively, and a significant decrease in the increase in HR to unilateral CPA-stimulation, by –40% & -56% respectively figure (9-A2&B2), indicated that the decrease was instead augmented under the influence of halothane.

Anesthetic Influence on α2-/I(1)-Receptors Mediated Clonidine Effects

A3&B3 and A4&B4 in figures (6,7,8,9) demonstrated respectively the effects of yohimbine (500pmol/50nl) and idazoxane (270nM), bilaterally administered 2-3min prior to injection of clonidine (30nM) by same route. Figure (6-A3&B3) demonstrates that yohimbine partially antagonizes the hypotensive effects of clonidine, and presence of no differential between the influence of urethane and halothane on these effects, since the raise of basal SBP by +16% (75±4.8mmHg to 97±4.4mmHg) in urethane, and by +18% (91±3.8mmHg to 117±5.2 mmHg) in halothane, was in the same range, in comparison with the before treatment value of urethane (120±4.5mmHg) or halothane (138±8.8mmHg). The results shown in figure (7-A3&B3), also demonstrates a partial reverse to the effect of clonidine on the increase in the SBP to CPA-stimulation, raising it from Δ+40±.2.9mmHg to Δ+65±4.1mmHg in urethane, and in halothane from Δ+25±1.9mmHg to Δ+31±2.4mmHg, in comparison with the before treatment value of urethane (Δ+96±4.9mmHg) or halothane (Δ+39±4.7mmHg).

The results shown in figure (8-A3&B3), demonstrates a partial reverse to the effect of clonidine on the basal HR by yohimbine that raised by+26% (210±8.8mmHg to 315±9.4mmHg) in urethane and by+29% (200±6.9mmHg to 300±6.9mmHg) in comparison with the before treatment in urethane (375±7.9mmHg) or in halothane (380±9.1mmHg). Also a partial reverse to the effect of clonidine was raised the increase in HR to CPA-stimulation from Δ+39±1.9mmHg to Δ+53±3.7mmHg in urethane, and from Δ+31±4.9mmHg to Δ+48±5.7mmHg in halothane, in comparison with the before treatment in urethane (Δ+65±4.9 mmHg) or halothane (Δ+70±4.6mmHg), as illustrated in figure (9-A3&B3).

Figure (6-A4&B4), on other hand, demonstrates that idazoxane completely antagonizes the hypotensive effects of clonidine, and presence of no differential between the influence of urethane and halothane on idazoxane reversing effect, since the raise in the basal SBP of urethane group was from 75±6.8 to 115±7.8mmHg in comparison with the before treatment value (120±4.5mmHg), and of halothane from 91±3.8mmHg to 131±5.4mmHg, in comparison with the before treatment value (138±8.8mmHg). Whereas figure (7-A4&B4), also demonstrates a complete reverse to the effect of clonidine on the increase in the SBP to CPA-stimulation, raising it from Δ+40±.2.9mmHg to Δ+88±8.9mmHg in urethane, and from Δ+25±1.9mmHg to Δ+33±4.2mmHg) in halothane, in comparison with the before treatment value of urethane (Δ96±4.9mmHg) or halothane (Δ39±4.7mmHg).

The reduction in the basal HR due to clonidine effect that shown in figure (8-A4&B4), was also completely antagonized by idazoxane in urethane (210±8.8mmHg to 360±11.9mmHg) and halothane (200±6.9mmHg to 355±9.1mmHg), and in the increase of HR to CPA-stimulation figure (9-A4&B4), from Δ+39±1.9mmHg to Δ+59±5.6mmHg in urethane, and from Δ+31±4.9mmHg to Δ+61±7.1mmHg in halothane, in comparison with the before treatment value Δ+96±4.9mmHg of urethane, or Δ+39±4.7mmHg of halothane. The complete reversing effect of idazoxane vs. partial of yohimbine to clonidine hemodynamic actions, suggested two indications, the first is the predominant role played by imidazoline receptors of RVLM in mediating the mechanism of clonidine hypotension, and the second is the presence of no anesthetic interference with the mechanism of clonidine action.

A5&B5 in figures (6,7,8,9) further illustrates the effects of yohimbine & idazoxane, bilaterally and sequentially administered 2-3min prior to intra-RVLM injection of clonidine. Under influence of urethane, the combined antagonists was completely abolished the hemodynamic effects of clonidine, and over and more reversed the blood lowering effect ($p<0.05$) of urethane (119±3.7mmHg) to a level (134±4.9mmHg) of no differences with that of basal BP of halothane (142±6.4) or control (139±5.8mmHg). The sequential injection of yohimbine+idazoxane in halothane rats however also completely abolished the hypotensive effects of clonidine (136±6.6mmHg), but did not go behind the before treatment level, indicated presence of differential influences for urethane vs. halothane on α2-RVLM mediating mechanism.

Anesthetic Influence on Central Antagonism of Peripheral Clonidine

To investigate further the pharmacological mechanism involved in the urethane hypotensive effect, peripherally administration of clonidine (100nmol/kg i.v.) in conscious normotensive rats, was hypotensive ($p<0.001$) by -31% of basal SBP (139±5.8mmHg). In urethane anesthetized rats, bilateral intra-RVLM injection of idazoxane (270nM), 2-3min following to i.v. administration of clonidine (100nmol/kg), completely abolished the hypotensive effect of clonidine to before treatment BP baseline (124±4.3mmHg), whereas sequential bilateral intra-RVLM injection of idazoxane+yohimbine, completely reversed the effect of clonidine and over and more raised the baseline of SBP to a level close to that of conscious normotensive rats (133±4.8mmHg), as illustrated in Table (1) and figure (10).

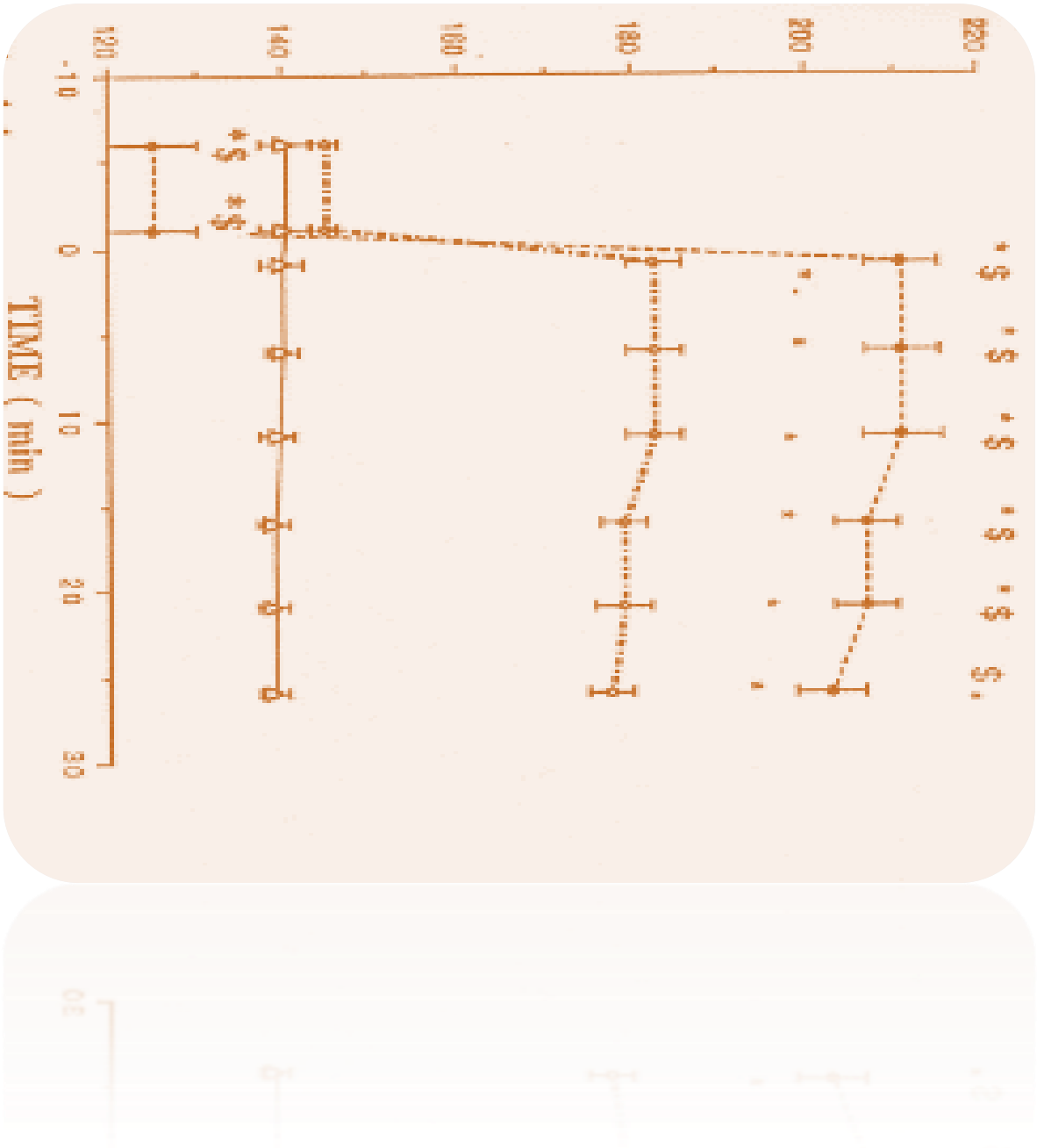


Fig.(4):Basal SBP, and increases in BP (mmHg) during 30 min electrical stimulation of the CPA region of urethane (●), halothane (◊) anaesthetized, and control rats received no anesthesia or stimulation (Δ), at the times -6 and -1 min before stimulation (basal BP), and 1, 6, 11, 16, 21, & 26min during stimulation. The comparison of the differences in the sequential values of the same group of rats (* P<0.001), or in the inter-groups values ($$$ P<0.001), 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.**

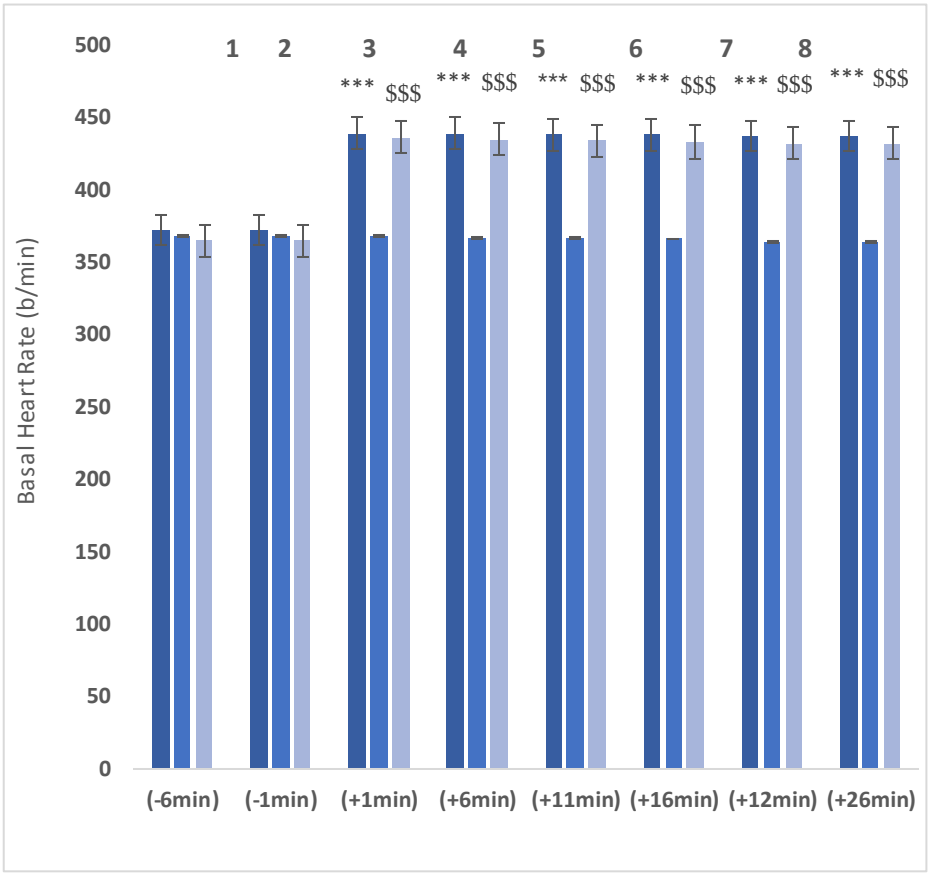
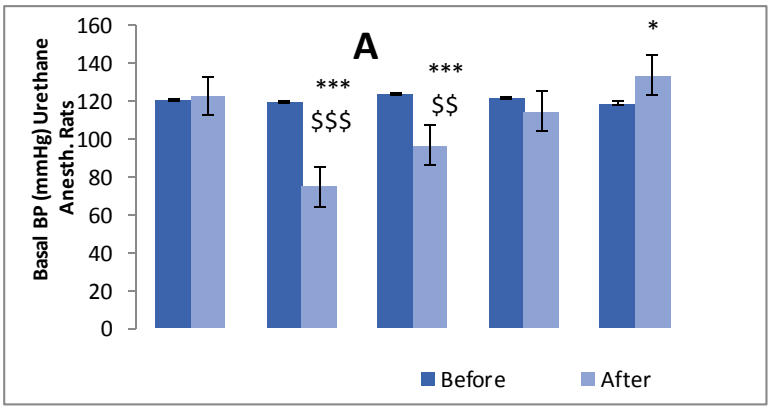


Fig. (5): Graphic demonstrating the basal HR, and the increases in HR (b/min) during 30 min electrical stimulation of the CPA region of urethane (dark columns), halothane (light blue columns) anaesthetized, and control (blue columns) rats received no anesthesia or stimulation (Δ), at the times -6 and -1 min before stimulation (basal BP), and 1, 6, 11, 16, 21, & 26min during stimulation. The differences between urethane (*** P≤0.001) or halothane ($^{\$\$\$}$P<0.001) effects, were compared using unpaired Student's t-test and paired Student's t-test. Values give mean ±S.E.Mof6rats/group.



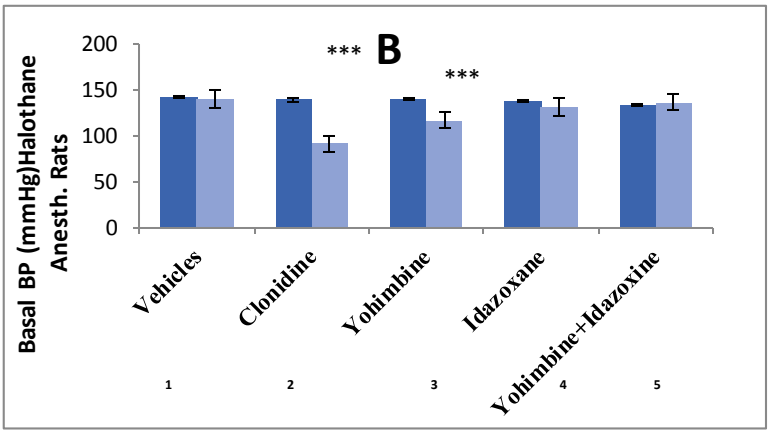


Fig. (6): Histogram showing the influence of urethane (A) or halothane (B) anesthetics on basal SBP (mmHg) before (blue columns) and after (light blue columns) intra-RVLM injection of vehicles, clonidine, clonidine antagonized with yohimbine, idazoxane, or yohimbine+ idazoxane. The results are expressed as mean ±S.E.M. of 6 rats/group.* P≤0.01, and ***P≤0.001, using paired Student's t-test comparing after with before treatment levels, or halothane ($^{\$\$}$P≤0.01 and $^{\$\$\$}$P≤0.001) effects were compared using unpaired Student's t-test.

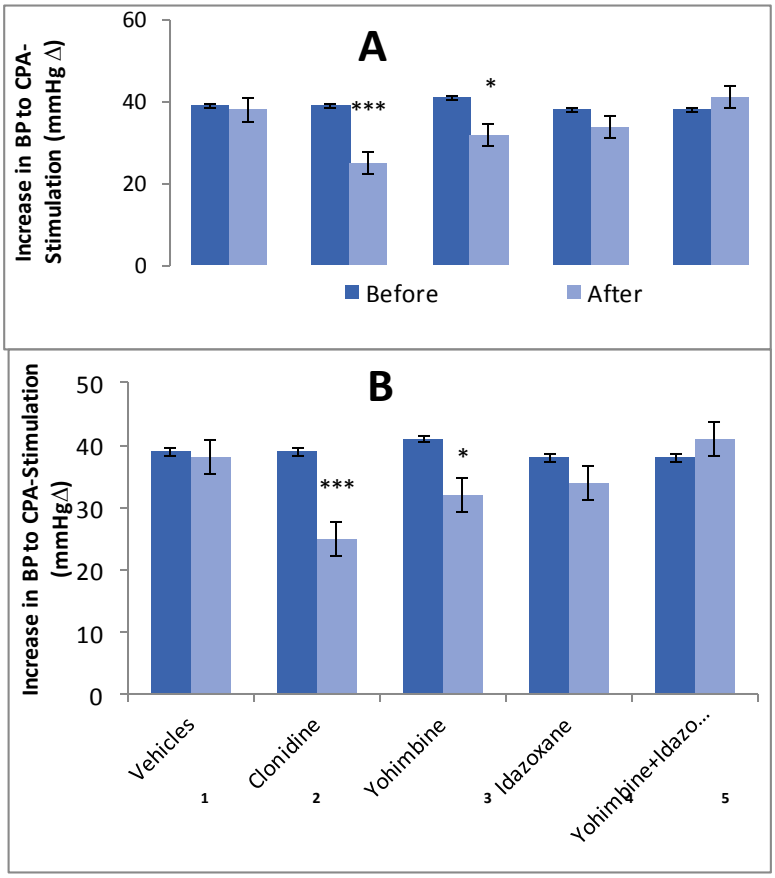


Fig. (7):

Histogram showing the influence of urethane (A) or halothane (B) anesthetics on the increase in basal SBP to CPA-stimulation (Δ mmHg) before (blue columns) and 15min after (light blue columns) intra-RVLM injection of vehicles, clonidine, clonidine antagonized with yohimbine, idazoxane, or yohimbine+ idazoxane. The results are expressed as mean ±S.E.M. of 6 rats/group. *P<0.05 and ***P<0.001, using paired Student's t-test comparing after with before treatment levels.

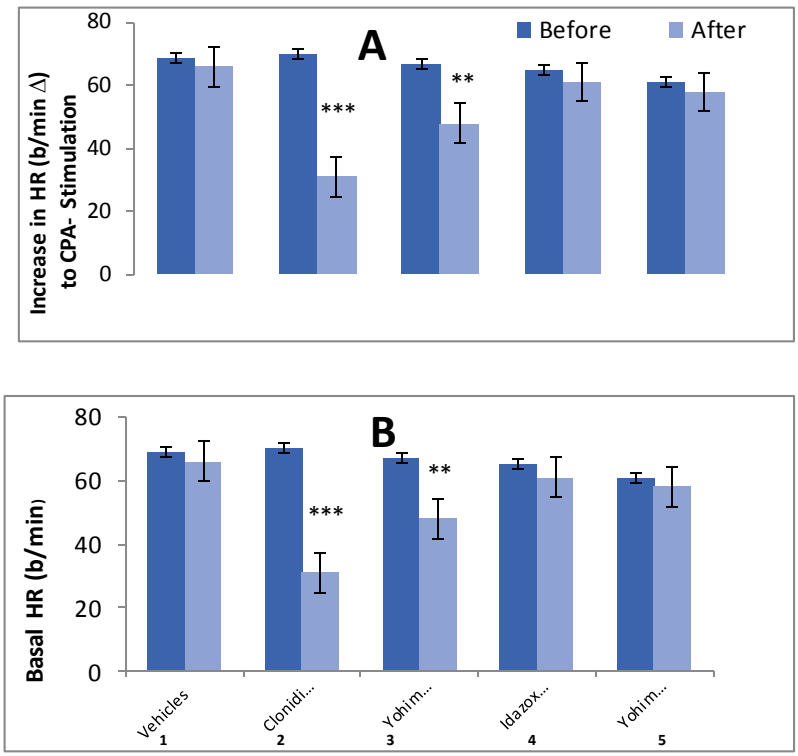


Fig. (8): **Histogram showing the influence of urethane (A) or halothane (B) anesthetics on basal HR (b/min) before (blue columns) and after (light blue columns) intra-RVLM injection of vehicles, clonidine, clonidine antagonized with yohimbine, idazoxane, or yohimbine + idazoxane. The results are expressed as mean ±S.E.M. of 6 rats/group. **P≤0.01 and ***P≤0.001 using paired Student's t-test comparing after with before treatment levels**

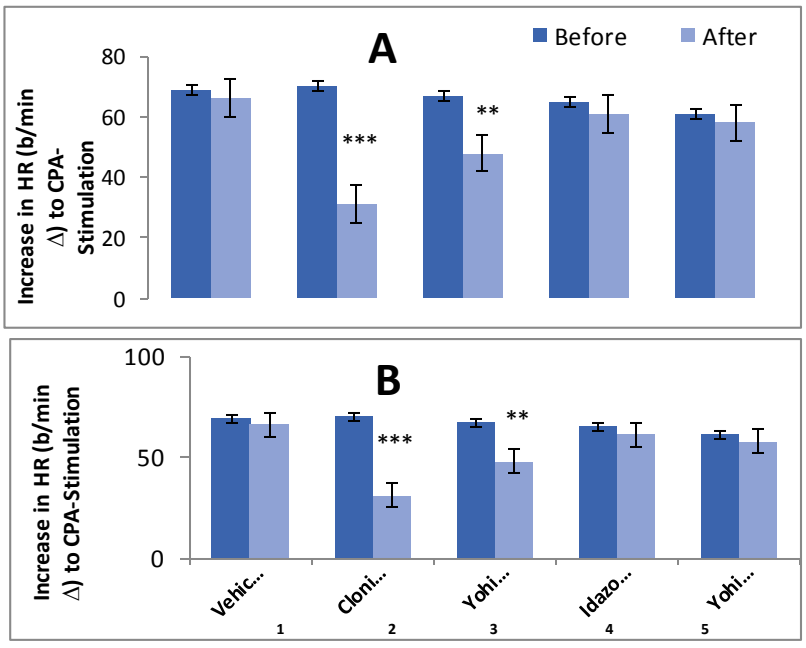


Fig. (9): Histogram showing the influence of urethane (A) or halothane (B) anesthetics on the increase in HR (b/min) to CPA-stimulation before (blue columns) and 15min after (light blue columns) intra-RVLM injection of vehicles, clonidine, clonidine antagonized with yohimbine, idazoxane, or yohimbine+ idazoxane. The results are expressed as mean ±S.E.M. of 6 rats/group. **P<0.01 and ***P<0.001, using paired Student's t-test comparing after with before treatment levels.

Table (1): Changes in SBP of conscious rats when i.p. injected with urethane (1.1mg/kg), followed by i.v. clonidine (100nmol/kg), before blockade with intra-RVLM injection of idazoxane (270nM) or sequential blockade with yohimbine (500nM)+idazoxane (270nM). The results are expressed as mean ±S.E.M. of 6 rats/group. * P≤0.05, using paired Student's t-test comparing after with before treatment levels, and ($P<0.05) unpaired Student's t-test comparing the value between groups.

Peripheral administratation	Intra-RVLM administration	Measurements	Systolic BP mmHg		Heart Rate beat /m	
			Before intra-RVLM Treatment	After Treatment	Before intra-RVLM Treatment	After Treatment
Vehicle (i.v.) (conscious)	-	Basal BP&HR	139±5.8	-	370±10.2	-
Urethane (i.p.)	Vehicle	Basal BP&HR.	124±4.3$	121±5.3	370±10.2	376±8.5
Pre-Vehicle (i.v Urethane(i.p)		CPA-stimul.Δ	+98±5.7	+96±5.1	+68±9.9	+71±9.3
Pre-clonidine i.v) (100nM)	Idazoxane	Basal BP&HR	78±4.3$	120±5.8*	375±9.6	360±9.9
		CPA-Stimul.Δ	+45±5.9$	+78±4.9*	+69±5.7	+67±6.6
	and Idazoxane+ yohimbine.	Basal BP&HR	122±3.9	133±4.8*	380±8.9	378±9.8
		CPA-Stimul.Δ	+88±6.4	+89±5.7	+68±3.3	+66±4.1

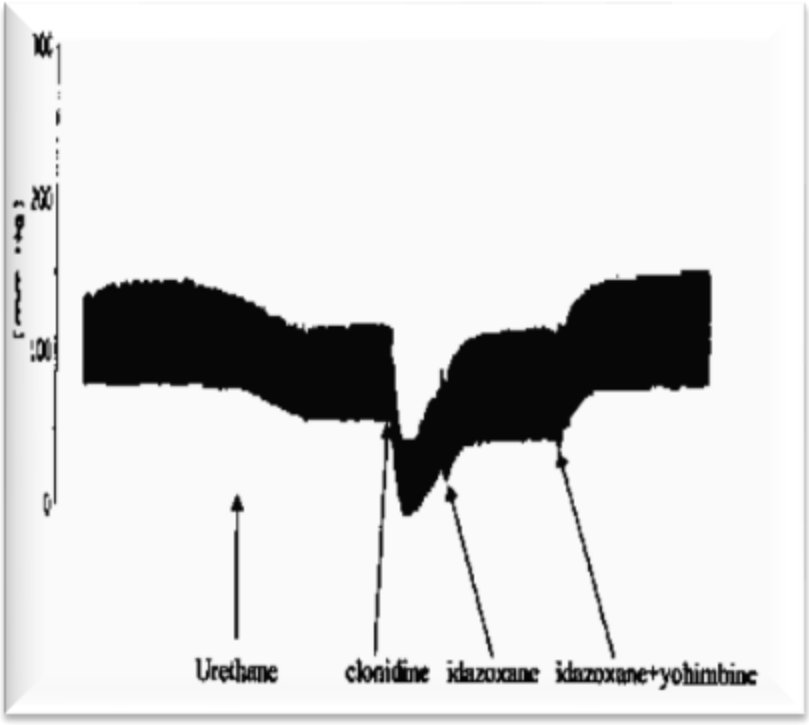


Fig. (10): Representative authentic recordings in Wistar rats, of SBP changes in conscious values following i.p. injection of urethane (1.1mg/kg), followed by i.v. clonidine (100nmol/kg), before blockade with intra-RVLM injection of idazoxane (270nM) or sequential blockade with yohimbine (500nM) idazoxane (270nM).

Discussion

This *in vivo* study demonstrates presence of differential effects between urethane and halothane on basal BP and the increase in BP to CPA-stimulation. Over and more, it's the first sought to illustrate the role of α2-adrenoceptors in mediating the associated hypotensive effect of urethane anesthesia, and to answer whether it occurs at a central limb and/or peripheral, by comparing the results of urethane anesthetized rats with that of the halothane anesthetized or the control rats.

First of all, it is important to evaluate, the protocol and procedure used in present study. The evaluation of the unilateral electrical stimulation for more than 30min, using stimulating parameters of a 10sec train of 1msec square wave pulse duration at 40Hz and 2V every 30s to the CPA region both of urethane and halothane anaesthetized rats, produced frequently dependent (data not shown) increase in SBP, and a frequency producing a submaximal increase in SBP was

selected and used in this study. The choice of frequency is consistent with the data of our previous study on RVLM stimulation [19]. These parameters resulted in a constant and reproducible presser peak response with the same intensity over the 30min stimulation both in urethane and halothane anaesthetized groups since the reductions of 9.9% (in urethane group) and 8.9% (in halothane group), in the increase of SBP during the period of CPA-stimulation, where not significant, whereas 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. Conversely, there were no observations of cardiovascular responses when the stimulation was applied to areas outside the CPA. This is consistent with data obtained by other studies, indicated that the increase was related to activation of neurons within the discrete CPA [3]. In the present study, the increase in BP following CPA-stimulation, in urethane anaesthetized rats was unrestrained and 242% greater than that observed with halothane anesthesia, suggesting that the increase in BP to CPA-stimulation is anesthetic-dependent. In halothane anaesthetized rats, the pressor response was in some way initially restrained and only improved after many attempts to change the position of the stimulating electrode within the CPA. This practical problem with halothane is consistent with the previous conclusion [8,20] 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 CPA-stimulation than that of halothane, might be clarified by the observations that the rostral VLM and caudal VLM neuronal activity are connected with and affected by stimulation of the CPA [3], and perhaps correlated to the differences in the effects of the two anesthetics on the baroreflex control of BP. In present study, the controlled hypertension by CPA stimulation for several minutes would be expected to affect RVLM-neuronal activity [21], and one explanation to what was being noticed by this study, of higher BP response to CPA-stimulation by urethane than by halothane, probably due to urethane sedative effect on α2-adrenoceptors within RVLM, which mediate the baroreflex function. In support of this explanation, is the finding that bilateral injection into RVLM, of a low dose of the clonidine or its-like drugs, has a beneficial effect on improving the baroreflex function, providing that activation of α2-adrenoceptors-dependent mechanisms [21, 22, 23, for review see 24], and/or activation of non-adrenoceptors-dependent [25], and the improvement in spontaneous baroreflex sensitivity, probably through parasympathetic activation hypertension [26,27].

With respect to BP, urethane but not halothane anaesthetized rats showed a decrease in basal SBP when compared with that of non-anaesthetized non-stimulated controls. Since the basal SBP of the halothane group was within the range of the resting SBP in conscious normotensive Wister rats, urethane in current study therefore, produced a hypotension. Except that urethane produced a hypotension in current study is inconsistent with the data demonstrating no effect of urethane on BP when compared to unanaesthetized controls (10), however, it is in a good agreement with several lines of studies which have shown a reduction in the basal BP of the rats [7,28]. One mechanism at the cellular level proposed for the urethane hypotensive effect in the intact animals [29] is interference with Ca^{++} availability for contractions of K^{+} depolarized vascular [30] and tracheal smooth muscle [31], by inhibiting mobilization of intracellular Ca^{++} pool(s) from tightly bound storage sites [7,32]. Interference with transmembrane Ca^{++} fluxes would not seem to be a factor since this only occurs at concentrations higher than required for anesthesia [7]. Another proposal mechanism is that urethane anesthesia inhibits cardiovascular responses that are mediated via peripheral and central α2 –adrenoceptors [8]. Further, urethane ability to block the peripheral pre- or post-synaptic α2–adrenoceptors, might produce a vasodilatation by preventing the vasoconstrictive effects of released or circulating catecholamine thereby causing hypotension. Bennett and Gardiner [33] demonstrated a significant fall in BP in the urethane-anesthetized Wister, Long Evans, and Sprague Dawley rats after administration of AVP-V1 antagonist. The extent, however of this fall was related to the level of basal BP with Sprague Dawley rats exhibiting significantly lower BP, and thus a lower fall in BP to AVP-V1 antagonist, than Long Evans and Wister rats. Urethane may also act through the CPA region in affecting the neuronal activity inside the RVLM, which proposes as one of basis for the ongoing tonic activity of the RVLM premotor neurons [3,34], providing that unilateral L-glutamate microinjection of L-glutamate into the CPA of conscious rats caused a dose-dependent increase in arterial pressure and respiratory rate which markedly attenuated by subsequent urethane anesthesia [35]. Urethane is commonly administered to induce surgical anesthesia in rats at doses 1.2-1.5 g/kg i.p., but consistent with the dose used in the present study lower doses (0.8-1.1 g/kg) have been found sufficient to induce a suitable level of surgical anesthesia [36].

The finding of a reductions in the basal SBP&HR, and in the increase in SBP&HR to CPA-stimulation following to bilateral intra-RVLM injection of clonidine after and 15min before 5min CPA-stimulation in one experiment, showed of no differential effects between the urethane and halothane, on the mediating role of RVLM-α2-/I(1)-receptors, suggested that neither urethane nor halothane interfered with the mechanism mediated the central hypotensive effects of

clonidine. Our findings are in a good agreement with the findings of many previous studies [2,21,22,24,25,37], which confirmed the hemodynamic response to clonidine to have been manifested by reduced arterial pressure, heart rate, and renal blood flow rate . However, presence of a differences ($p \leq 0.01$) in the levels of the basal BP in urethane (75mmHg) versus halothane (91mmHg), and in the increase in SBP to CPA-stimulation in urethane (-58%) versus halothane (-36%) anesthetics, expressed different central mechanism of action of each in maintaining of the hemodynamics, although it's in the favorite of urethane [38]. Several line of studies has concluded that RVLM is the primary site of action for a centrally acting antihypertensive, clonidine. In this region, an argument on a differential mechanisms for clonidine and clonidine like-drugs hemodynamic actions, have been proposed by the investigators [for review see 24], on whether it is predominantly mediated through alpha-2 adrenergic receptors [30] or through specific imidazole receptors site [50,51,52], or even through the both α2-/I(1)-sites [6,21,42]. In consistent with the first of these proposals, was the findings that microinjection into RVLM of yohimbine prior to intra-RVLM injection of clonidine, did not modify BP and HR, but antagonizes partially the hemodynamic effects of clonidine at their central site of α2-adrenoceptor sites in both anesthetic groups, whereas the microinjection into RVLM of idazoxane prior to intra-RVLM injection of clonidine, antagonizes completely the hemodynamic effects of clonidine at their central I(1)-imidazoline receptor sites in both anesthetic groups. However, the finding that idazoxane + yohimbine, completely abolished the lowering effect of clonidine on basal HR regardless the influence of the used anesthetics, may indicated a synergism role between α2-adrenergic- and nonadrenergic I(1)-imidazoline-receptors, in regulation of the hemodynamic parameters , which is consistent with the third of the above proposals. Several mechanisms of action have been proposed for a hypotensive effects induced by injection into the RVLM of clonidine or clonidine-like drug, moxonidine, in SHR. Enhanced baroreflex sensitivity control of sympathetic activity and HR, have been proposed in one mechanism to be mediated via an imidazol receptors and not the α2-adrenoceptors [26], or to be via contribution of the both I(1)/α2-receptors in rabbits [23], or to be via parasympathetic activation in conscious mice [27], whereas conflicting findings have been noticed in normotensive models [23]. However, involvement of mechanism at the RVLM, other than I(1)/α2-receptors, in decreases BP, HR, and sympathetic activity, and modulation of the baroreflex control of HR, have been suggested to be mediated probably through involvement of ANG II and ANG-(1-7) [43], and AMPA/kainite receptors [25].

The strike result of Yohimbine and idazoxane administered Sequentially following clonidine injection into RVLM, in the of urethane anesthetized rats, was not only their complete reverse of the hypotensive effects both of clonidine and urethane, but also raising over and more the basal BP from hypotensive level of urethane to the level of the control. The data of this experiment suggested; first, a possible interaction between α2-adrenoceptors and I1-imidazoline receptors in antagonizing the hypotensive effects of clonidine within the RVLM regardless the used anesthetic. Second, it also suggests an implication of central α2-adrenoceptors in mediating the hypotensive effect of urethane. The hypothesis of possible synergism between α2- and I (1)-receptors in reflex and tonic control of the sympathoexcitatory premotor neurons of the RVLM, that may give support to the results of this study, have been proposed by several studies [6,23,42], which suggests that alpha2-adrenoceptor hypotension can be produced in the RVLM, possibly as a result of activating imidazoline receptors [23].

The complete antagonism of centrally administered idazoxane to the hypotensive effects of periphery administered clonidine in urethane anesthetized rats, suggested involvement of central I(1)-imidazole receptors in preventing of the hypotension produced only by clonidine, but not that produced by urethane, since the antagonism only raised the BP baseline from 77±5.3mmHg to 113±5.8mmHg. However, sequential central administration of yohimbine+idazoxane rose over and more the baseline of the BP to the level close to that of conscious normotensive rats. These finding suggested implication both of peripheral and central α2-adrenoceptors in the mechanism of action of clonidine and urethane anesthesia.

In conclusion, the results of this study proposed that *investigation of α2-/I (1)-receptors agonist, clonidine,* no matter the anesthetic used, is an attractive tool for understanding of the pharmacological base of the mechanisms surrounding anesthetic action and consciousness. This study analyzed pharmacologically the role of peripheral and central (RVLM) α2-/I(1)-receptors in mediating the hemodynamic effects of clonidine or urethane anesthesia, in comparison with that in halothane anesthesia or control groups, and many conclusions are drawn. The first is that the sympathoinhibitory effects of clonidine or urethane is best explained by activation of both α (2)-/I (1) receptors. The second is that both of α2-adrenoceptors the peripheral and the central are involved in mediating of the hypotensive effect of urethane anesthesia.

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