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The Dual Cardiovascular Effect of Centrally Administered Clonidine: A Comparative Study between Pentobarbital- and Ketamine/Xylazine-Anesthetized Rats

Natália Kimie Matsubara and José Eduardo da Silva-Santos *

Laboratory of Cardiovascular Biology, Department of Pharmacology, Universidade Federal de Santa Catarina, Florianópolis 88040-900, SC, Brazil; matsubara.natalia@gmail.com
* Correspondence: j.e.silva.santos@ufsc.br

Abstract: The administration of the α_2-adrenergic receptor agonist clonidine via intracerebroventricular route produces hypotension in pentobarbital-anesthetized rats and pressor responses in conscious normotensive rats. We explored the impact of different anesthetics on the central nervous system-dependent cardiovascular effects of clonidine. Normotensive male Wistar rats with guide cannulas previously implanted in the cerebroventricular system were anesthetized with pentobarbital or ketamine/xylazine and prepared for blood pressure measurement. The animals received intracerebroventricular injections of 10 µg clonidine or 0.6 µg dexmedetomidine, and the effects on the systolic, diastolic, mean arterial pressure, and heart rate were evaluated. The influence of 5 µg yohimbine, a selective α_2-adrenergic receptor antagonist, was also assessed. The i.c.v. microinjection of clonidine decreased all three components of systemic arterial pressure and the heart rate of pentobarbital-anesthetized rats. On the other hand, clonidine increased the blood pressure and generated a less intense reduction in the heart rate of ketamine/xylazine-anesthetized rats. The pressor and bradycardic effects of clonidine in ketamine/xylazine-anesthetized animals were reproduced by dexmedetomidine, a more selective α_2-adrenergic receptor agonist. Notably, the previous intracerebroventricular injection of yohimbine significantly inhibited the hypertensive effect of clonidine and dexmedetomidine. This study discloses that while normotensive rats anesthetized with pentobarbital show hypotensive responses, the stimulation of α_2-adrenergic receptors increases the blood pressure in rats under ketamine/xylazine-induced anesthesia, reproducing the effects seen in conscious normotensive animals. Recognizing the mechanisms involved in these differences may allow us to understand better the final effects of clonidine and other α_2-adrenergic receptor agonists in the central nervous system, contributing to the repurposing of these drugs.

Keywords: anesthetic combination; cardiovascular responses; sympathetic; presynaptic receptors

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1. Introduction

Clonidine is a well-known agonist of both α_2-adrenergic receptors (ARs) and imidazoline receptors, as demonstrated in several experimental approaches; for review, see [1]. Clonidine can also act as a partial agonist at α_1-ARs [2] and has been associated with either stimulatory or inhibitory effects on multiple targets, including but not limited to ion channels [3,4], ion-channel-coupled receptors [5], and solute carrier proteins [6]. Clonidine induces α_2-ARs-dependent contractile responses in arteries [7] and veins [8]. Nevertheless, the systemic administration of clonidine in humans was early associated with hypotensive and antihypertensive actions [9], an effect entirely dependent on the central nervous system [10]. Indeed, the principal cardiovascular effects of clonidine are putatively associated with the activation of presynaptic α_2-ARs in the brainstem, which, once stimulated, reduce norepinephrine release, depressing the sympathetic tone and lowering both blood pressure and heart rate [11].

Clonidine was granted U.S. Food and Drug Administration approval for hypertension in 1974 [12], and it remains an alternative for patients suffering from resistant hypertension [13]. In addition, it has been successfully used to treat attention deficit hyperactivity disorder [14] and pain [15]. Moreover, clonidine has several off-label uses, such as the treatment of anxiety, withdrawal symptoms of alcohol and opiates, and other psychiatric conditions [16]. Notably, despite α_2-ARs having been implied in all these processes, the exact mechanism by which clonidine exerts its effects remains to be elucidated. The potential effectiveness for some untreatable conditions allied with the safety of this medicine reinforces the need for continuous efforts to disclose the mechanistic details of clonidine's effects.

Although multiple studies have reproduced the antihypertensive effect of clonidine, a closed analysis of the literature reveals that such findings are strikingly dependent on experimental conditions. The administration of clonidine using the intracerebral route decreased the blood pressure of both conscious hypertensive [17] and anesthetized animals [18–22]. However, despite its efficacy against hypertension, the i.c.v. administration of clonidine in conscious normotensive rats resulted in the opposite effect, increasing blood pressure [23–25].

Anesthetic agents can have a profound impact on experimental animal models. Thus, when the role of the central nervous system is directly explored, including in cardiovascular research, careful anesthetic selection must be carried out. The anesthetics used in the previously mentioned studies demonstrating the hypotensive effect of clonidine were either pentobarbital or urethane. Pentobarbital is a barbiturate that binds and enhances the activity of ion-channel-coupled gamma-aminobutyric acid type A ($GABA_A$) receptors, resulting in a generalized central nervous system depression [26]. The modulation of $GABA_A$ receptors by pentobarbital impacts the activity of cardioinhibitory pathways [27], an effect that may directly alter central nervous system-mediated clonidine responses. Thus, the divergence between the effects of clonidine in conscious and anesthetized animals can directly result from the use of the allosteric modulators of $GABA_A$ receptors as anesthetic agents. In recent decades, the ketamine/xylazine combination has been a common anesthetic choice for experimental animal studies due to minimal effects on the cardiovascular and respiratory systems, possibly without any interaction with the GABAergic pathway [28].

We designed this study to explore the impact of two different anesthetic protocols (pentobarbital and ketamine/xylazine) on the central nervous system-dependent cardiovascular effect of clonidine. Because the putative impact of ketamine and xylazine on cardiovascular function is reduced compared to other drugs, we hypothesized that normotensive rats subjected to this anesthetic combination would present the same hypertensive profile of responses described for conscious animals subjected to centrally administered clonidine. Understanding how anesthetics modulate the cardiovascular effects of clonidine can allow us to further harness α_2-adrenergic receptor ligands as pharmacological tools, as well as for central nervous system disorders.

2. Materials and Methods

2.1. Animals

Male Wistar rats weighing 300–350 g supplied by the Central Vivarium of Universidade Federal de Santa Catarina (Florianópolis, SC, Brazil) were kept at a controlled temperature (22 $\pm$ 1 °C), with a 12/12 h light/dark cycle and free access to food and water. This study was approved by the Animal Care and Use Committee from Universidade Federal de Santa Catarina (6327180718). All surgical procedures were performed under general anesthesia and started only when the animals were unresponsive to pinch stimuli on their tails and pelvises. When necessary, anesthetics were supplemented with 30% of the initial dose. A total of 56 male rats were required to complete this study, including those needed for preliminary experiments and losses during anesthesia and surgical procedures. The animals were randomly divided between the experimental groups described ahead.

2.2. Stereotaxic Surgery

Stereotactic guidance was used to perform injections into the lateral cerebral ventricle. Briefly, the animals were anesthetized with ketamine and xylazine (100 and 20 mg/Kg, i.p., respectively) and placed in a stereotaxic frame (Stoelting Co., Wood Dale, IL, USA). A stainless steel guide cannula (23 gauge; 11 mm long) was implanted 1 mm above the lateral ventricle using the following stereotaxic coordinates: anteroposterior = −0.8 mm from bregma; lateral = −1.5 mm from sagittal suture; and vertical = −3.6 mm deep from skull. The guide cannula was fixed in the skull with dental cement. After the surgery, the animals were allowed to recover for three days before blood pressure measurements.

2.3. Intracerebroventricular (i.c.v.) Drug Administration

All i.c.v. microinjections were performed with a thin needle (185 μm outside diameter, 33 gauge) introduced through a guide cannula until its tip was 1.0 mm below the cannula end. The drugs were dissolved in sterile phosphate-buffered saline (PBS) and were injected in 5 μL final volume using a hand-driven microsyringe (10 μL, Hamilton Co., Reno, NV, USA) connected to the 33 G injection needle by a polyethylene catheter (PE 10). The flow was carefully controlled by checking an air bubble inside the tube. The injection needle was left in place for 1 min after each i.c.v. administration to avoid the reflux of the administered volume.

2.4. Assessment of Systemic Blood Pressure

As described in the next section, the blood pressure was assessed in rats anesthetized with either ketamine/xylazine or pentobarbital. After the animals reached the surgical plane of anesthesia, an incision was made in the ventral cervical area to expose the right carotid artery, which was isolated from the vagus nerve to insert a previously heparinized 22-gauge catheter. The catheter was fixed in the artery and connected to a pressure transducer, allowing continuous recording of the systolic and diastolic pressures (SAP and DAP, respectively; in mmHg) and heart rate (in beats per minute, bpm) using a computer data acquisition system and software (PowerLab 4/30 and LabChart version 7.3; both from AD Instruments, Castle Hill, Australia).

2.5. Comparison of the Cardiovascular Effects of Centrally Administered Clonidine in Ketamine/Xylazine and Pentobarbital-Anesthetized Rats

In the first set of experiments, we assessed the blood pressure and the effects of the i.c.v. administration of clonidine in groups of animals anesthetized with either sodium pentobarbital (50 mg/Kg, i.p.) or the combination of ketamine and xylazine (100 and 20 mg/Kg, respectively, i.m.). Since preliminary experiments revealed a high rate of losses among pentobarbital-anesthetized rats, putatively associated with respiratory depression, we performed this set of experiments in animals under mechanical ventilation (VentElite 55-7040, Harvard Apparatus, Holliston, MA, USA; respiratory rate: 60 breaths per minute; tidal volume: 7 mL/Kg; positive end-expiratory pressure: 2 cmH_2O).

After a resting period following the surgical manipulation, the basal systemic arterial pressure and heart rate values were recorded for 20 min. Then, an i.c.v. microinjection of clonidine (10 μg/5 μL) was administered as described before, and the effects on blood pressure and heart rate were registered for up to 100 min. The experimental setup and timeline of intracerebroventricular injections adopted in this study are summarized in Figure 1.

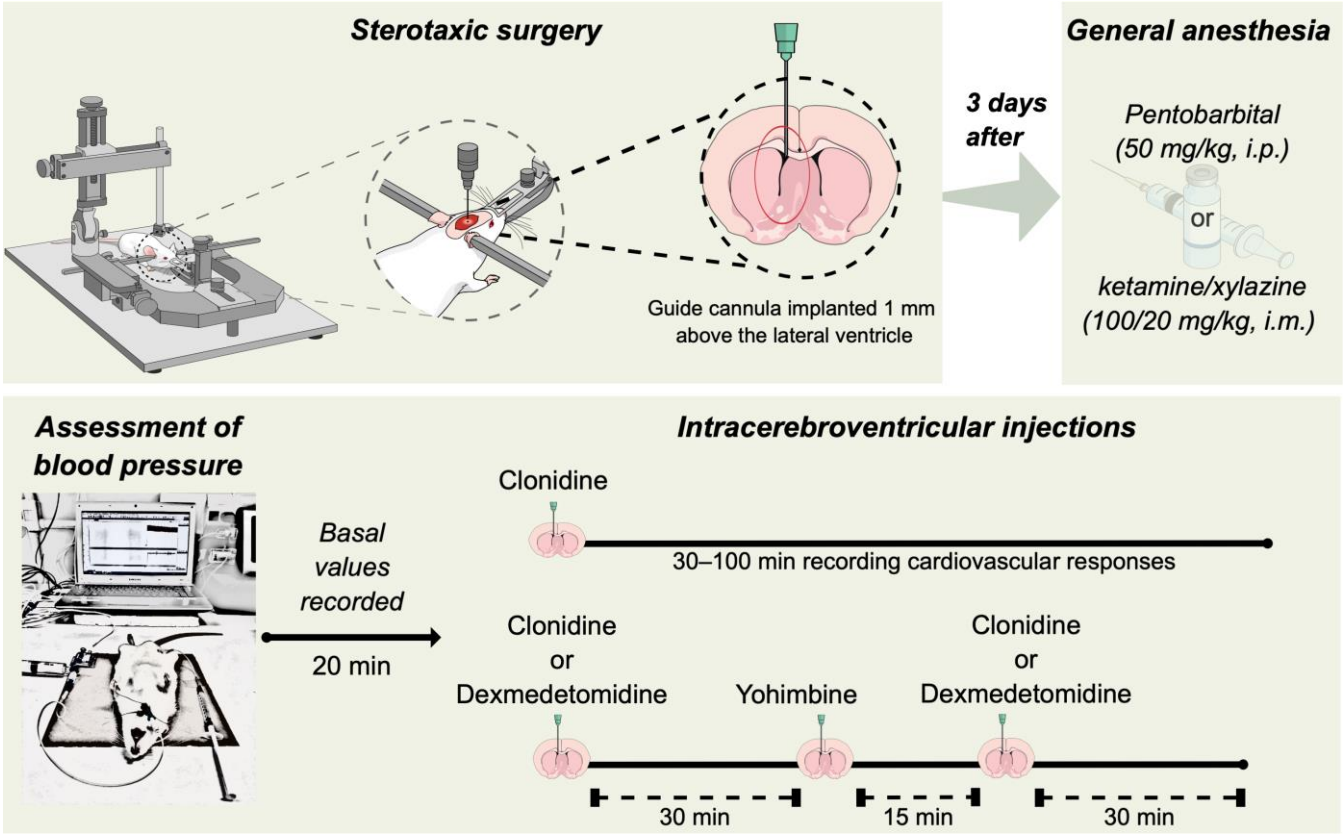

Figure 1. Summary of the experimental setup and schematic representation showing the timeline of intracerebroventricular injections performed in this study. All animals underwent stereotaxic surgery when a guide cannula was implanted to allow intracerebroventricular injections. Three days later, the animals were randomly anesthetized with either pentobarbital or a combination of ketamine/xylazine and were surgically prepared for direct blood pressure assessment. The basal arterial pressure and heart rate were recorded for 20 min before any drug injection. After that, groups of pentobarbital- or ketamine/xylazine-anesthetized rats received a single intracerebroventricular injection of clonidine, and the cardiovascular responses were recorded at 30–100 min. In a separate set of experiments, groups of ketamine/xylazine-anesthetized rats received intracerebroventricular injections of either clonidine (10 μg) or dexmedetomidine (0.6 μg), followed by yohimbine (5 μg), and either clonidine or dexmedetomidine again (refer to the timeline inside the figure for the intervals between the administrations). The animals were euthanized by anesthetic overdose at the end of these protocols. Evans blue dye (5 μL of 1%) was injected through the guide cannula, and the brain was removed to evaluate the diffusion of the dye in the ventricle. All rats used in our study had correct guide cannula placement. This scheme was created with a personal subscription to the Mind the Graph platform (www.mindthegraph.com).

2.6. Evaluation of the Role of α_2-Adrenoceptors in the Pressor Effect of Clonidine

Only animals subjected to the ketamine and xylazine anesthetic combination were used in the second set of experiments. The animals in this set of experiments were prepared for blood pressure assessment as described before but were allowed to breathe spontaneously. After the cannulation of the carotid artery, the 20 min interval of rest, and the recording of the basal systemic arterial pressure and heart rate, separated groups received an i.c.v. microinjection of clonidine (10 μg/5 μL) or dexmedetomidine (0.6 μg/5 μL), a more selective α_2-AR agonist. The effects of clonidine and dexmedetomidine on blood pressure and heart rate were monitored for 30 min. Then, the animals were treated with an i.c.v. injection of the selective α_2-adrenoceptor antagonist yohimbine (5 μg/5 μL). Fifteen min later, a second i.c.v. injection of clonidine or dexmedetomidine was administered, and the blood

pressure and heart rate were re-evaluated for 30 min (see Figure 1 for a schematic diagram including the sequence of i.c.v. injections). All animals were euthanized with an anesthetic overdose at the end of the assays. We injected 5 µL of 1% Evans blue dye to validate the injection site through the i.c.v. cannula and removed the brain to evaluate the diffusion of the dye in the ventricle. All rats in our study had correct guide cannula placement, as confirmed with the macroscopic analysis of the region stained with Evans blue.

2.7. Drugs and Solutions

Evans blue (#E2129), clonidine chloride (#C7897), yohimbine chloride (#Y3125), and all salts used to prepare the sterile phosphate-buffered saline (PBS; concentration in mmol/L: 137 NaCl, 2.7 KCl, 1.5 KH_2PO_4, and 8.1 $NaHPO_4$) were purchased from Sigma-Aldrich (Saint Louis, MO, USA). Dexmedetomidine hydrochloride (#AB120767) was obtained from ABCAM (Cambridge, UK). Heparin (Hemofol®; 5000 UI/mL) was obtained from Cristália (São Paulo, SP, Brazil). Ceva (Paulínia, SP, Brazil) supplied sodium pentobarbital, ketamine, and xylazine. All solutions and drugs were prepared and dissolved before each experiment.

2.8. Data Presentation and Statistical Analyses

All experimental groups included 5–6 different animals, as described in the legend of figures. Results were presented as the mean ± standard error of the mean, scatter plot, or individual values before and after the treatment with yohimbine, all indicating either the basal values or the effect of drugs on the SAP, DAP, the derived mean arterial pressure (MAP), and the HR of the animals. Statistical analyses were performed and figures were created using GraphPad Prism version 9.1.0 (GraphPad Software, San Diego, CA, USA). The results were subjected to unpaired or paired Student's *t*-test or one- or two-way analysis of variance (ANOVA) with repeated measures followed by Dunnet's or Šidák's post-tests, as appropriate. A significance level of 5% ($p < 0.05$) was considered for all applied tests.

3. Results

3.1. The Cardiovascular Effects of Intracerebroventricularly Injected Clonidine Depending on the Anesthetic Choice

We found no differences between the blood pressure of animals subjected to pentobarbital or ketamine/xylazine anesthetic protocols before the i.c.v. administration of clonidine. For instance, the basal MAP was 107.2 ± 8.2 and 101.4 ± 7.9 mmHg in pentobarbital- and ketamine/xylazine-anesthetized animals, respectively (see Supplementary Table S1 for additional values). However, the HR was significantly lower in ketamine/xylazine-anesthetized rats than in the pentobarbital group ($p < 0.001$; Supplementary Table S1).

Notably, if, on the one hand, the i.c.v. injection of clonidine reduced the blood pressure of animals anesthetized with pentobarbital (for instance, SAP was decreased by 54.0 ± 9.0 mmHg; $p < 0.01$), on the other hand, clonidine increased the blood pressure of ketamine/xylazine-anesthetized rats (for instance, SAP was increased by 35.1 ± 7.0 mmHg; $p < 0.01$). The effects of clonidine on the blood pressure of animals anesthetized with pentobarbital or ketamine/xylazine can be visualized in the trace recordings shown in Figure 2A,B and are depicted in Figure 2C–H (see also Supplementary Figure S1 for crude values of blood pressure before and after clonidine administration in both groups). The hypotensive effect of clonidine in animals anesthetized with pentobarbital, as measured in the SAP, DAP, and MAP, lasted around 100 min. In contrast, the increase in the SAP, DAP, and MAP generated by clonidine in ketamine/xylazine-anesthetized rats remained significant for a shorter period (up to 25 min; Figure 2C,E,G, respectively).

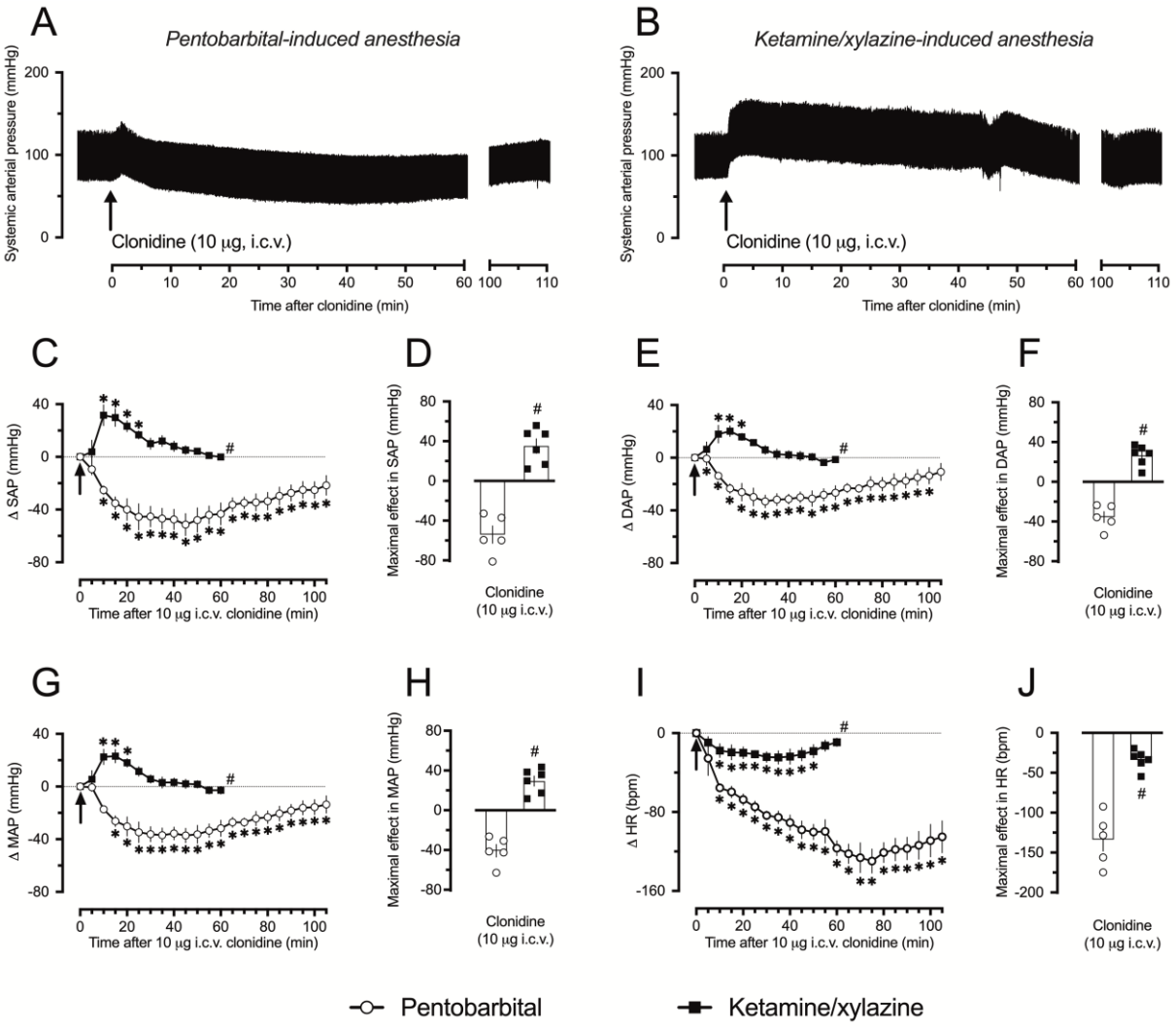

Figure 2. Differential influence of pentobarbital- and ketamine/xylazine-induced anesthesia on the cardiovascular effects of centrally administered clonidine in rats. Trace recordings showing the effects of 10 μg clonidine injected into the cerebroventricular system (i.c.v. pathway, indicated by arrows) on the blood pressure of rats under general anesthesia induced by pentobarbital (**A**) or ketamine/xylazine (**B**). The time course and maximal variation of systolic (SBP; (**C**,**D**)), diastolic (DBP; (**E**,**F**)), mean arterial pressure (MAP; (**G**,**H**)), and heart rate (HR; (**I**,**J**)) were evaluated over 30 or 100 min after clonidine injection, depending on the length of effects. All animals included in this set of experiments were maintained under mechanical ventilation. The results are presented as the mean ± standard error of the mean and the dot plot of data obtained from 5–6 animals per group. * indicates $p < 0.05$, compared with the basal values of the respective group before clonidine administration; # indicates $p < 0.05$, compared with the pentobarbital group. As appropriate, statistical analysis was performed using two-way ANOVA followed by Dunnet's or Šidák's post-tests or unpaired Student's *t*-test.

Regarding the cardiac responses, the i.c.v. administration of clonidine reduced the heart rate of both pentobarbital- and ketamine/xylazine-anesthetized groups. However, the drop in cardiac frequency was much more intense and lasting in those animals that received pentobarbital (Figure 2I,J). The numerical values of the effects of clonidine in these experimental groups are presented in Supplementary Table S2 for detailed comparisons.

3.2. The Pressor Responses to Clonidine in Ketamine/Xylazine-Anesthetized Rats Involve α_2-ARs

The ability of clonidine to increase blood pressure was reproduced in ketamine/xylazine-anesthetized animals under spontaneous breath (Figure 3A, black symbols), despite the reduced effect on the heart rate when compared with data from mechanically ventilated rats (compare the black symbols from Figures 3C and 2I). Similar to clonidine, the i.c.v. administration of dexmedetomidine in these animals also increased the blood pressure and showed a more prominent effect on the heart rate (Figure 3D–F, black symbols).

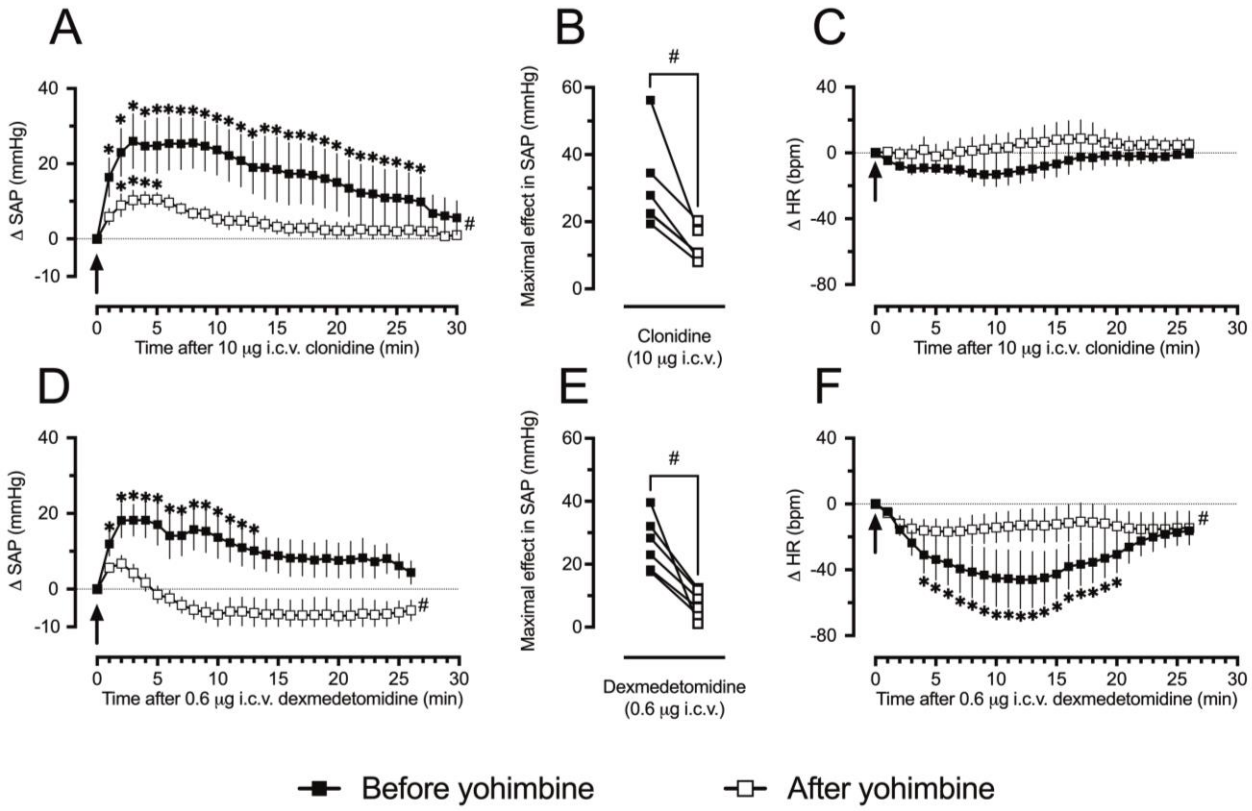

Figure 3. Impact of yohimbine on the cardiovascular effect of centrally administered clonidine and dexmedetomidine in rats subjected to ketamine/xylazine-induced anesthesia. Time course of variation in the systolic arterial pressure (SAP, (**A**,**D**)) and heart rate (HR, (**C**,**F**)) induced by the intracerebroventricular (i.c.v.) injection of clonidine (upper panels) or dexmedetomidine (bottom panels), as indicated by the arrows; in these experiments, the effects of clonidine and dexmedetomidine were measured before (black squares) and after (white squares) the i.c.v. administration of 5 μg yohimbine. The influence of yohimbine in the clonidine- or dexmedetomidine-increased SAP is also presented as individual values ((**B**,**E**), respectively). All animals included in this set of experiments were allowed to breathe spontaneously. The results show the mean ± standard error of the mean or individual values obtained in 5–6 animals per group. * indicates $p < 0.05$, compared with the basal values of the respective group before the administration of clonidine or dexmedetomidine; # indicates $p < 0.05$ compared with the responses recorded before yohimbine administration, at least between the intermediary points of the curves. As appropriate, statistical analysis was performed using two-way ANOVA followed by Dunnet's or Šidák's post-tests or paired Student's *t*-test.

The treatment of ketamine/xylazine-anesthetized rats with a single i.c.v. injection of yohimbine evoked a slight and transient fall in blood pressure without influencing the heart rate. For instance, the drop recorded in the MAP reached the maximal of 12.5 ± 3.3 mmHg and lasted less than 15 min ($p < 0.05$). Notably, yohimbine administration decreased the length and the peak of the pressor response to clonidine (Figure 3A,B). The treatment with yohimbine also prevented any significant effect of i.c.v.-administered dexmedetomidine

on blood pressure (Figure 3D,E). Interestingly, yohimbine did not significantly alter the already reduced effect of clonidine on the heart rate but significantly reduced the cardiac depression induced by dexmedetomidine (Figure 3C,F, respectively). The maximal effects of clonidine and dexmedetomidine on arterial pressure (SAP, DAP, and MAP) and HR and the influence of yohimbine on such effects are presented in Supplementary Table S3. Notably, the i.c.v. injection of 5 µL sterile PBS (the vehicle for all drugs used in this study) did not affect the cardiovascular parameters evaluated nor the pressor effects of clonidine and dexmedetomidine.

4. Discussion

Clonidine was launched as an antihypertensive drug in the 1970s, and it remains a third-line agent for the multidrug management of resistant hypertension. The final effect of clonidine in humans with hypertension is a centrally dependent blood pressure reduction. This action is putatively associated with α_2 presynaptic receptor stimulation in the cardiovascular regulatory center at the medullary portion of the brainstem. Briefly, the primary sympathetic neurons responsible for cardiovascular stimulation are in the rostral ventrolateral medulla (RVLM), while parasympathetic neurons accountable for the cardioinhibitory activity are in the nucleus ambiguous. The sympathetic neurons of the RVLM are tonically activated and are responsible for the post-ganglionic release of norepinephrine into peripheric neurons; for review, see [29]. Most RVLM neurons release norepinephrine or glutamate [30]. When α_2 presynaptic receptors are stimulated in RVLM neurons, the release of these neurotransmitters is reduced, and the efferent activity of the sympathetic system on the cardiovascular system is depressed, resulting in an immediate vasodilation and a decreased heart rate.

Clonidine directly microinjected in RVLM evokes hypotensive and sympathoinhibitory effects in anesthetized [31–33] and conscious [34] rats. Importantly, binding assays demonstrated that clonidine binds in the RVLM of rats [35], and several studies showed that blocking adrenergic or imidazoline receptors in RVLM inhibits the systemic hypotensive effect of clonidine, reviewed by [36]. Nonetheless, multiple experimental data strongly suggest that the neural mechanisms underlying the effects of clonidine on vascular and cardiac functions are more complex and do not involve the effects only on RVLM. A particularly intriguing finding is the paradoxical hypotensive or hypertensive effect exerted by clonidine when administered throughout the i.c.v. route into the central nervous system of pentobarbital-anesthetized or conscious animals, respectively.

In the present study, pentobarbital-anesthetized rats presented reduced arterial pressure and heart rate response to a bolus i.c.v. infusion of clonidine, corroborating the data of original studies using similar experimental conditions [10]. On the other hand, clonidine evoked pressor responses and bradycardia when administered into the central nervous system of ketamine/xylazine-anesthetized rats, reproducing data obtained in studies using conscious normotensive rats [24]. It was previously demonstrated that the hypotensive effect of both i.v.- [37] and i.c.v.- administered [21] clonidine was significantly potentiated by pentobarbital anesthesia in rats, circumventing the previously mentioned vasoconstrictor effect of clonidine. Clonidine has been administered as premedication or during anesthesia in humans, and its use has been associated with multiple benefits, including reduced anesthetic requirement [38] and improved hemodynamic parameters [39]. However, the cardiovascular effects of clonidine on blood pressure and cardiac function have not been explored experimentally under anesthesia induced by drugs other than pentobarbital-type agents. To our knowledge, this is the first study demonstrating that the i.c.v. administration of clonidine in rats anesthetized with the ketamine/xylazine combination results in augmented blood pressure, resembling the hypertensive responses found in conscious normotensive rats.

The mechanisms involved in ketamine/xylazine-induced anesthesia largely depend on the blockade of NMDA receptors by ketamine. However, ketamine has been associated with several targets and modulatory effects in the brain, which account for its immediate

and delayed neuropharmacological actions; for review, see [40]. Ketamine has been used as a sedative and anesthetic agent for humans since the 1960s, and its usage has increased in the last couple of decades. Like any other intravenously administered anesthetic, ketamine is subjected to substantial side effects and limitations, but it is recognized for its reduced risk of respiratory and hemodynamic depression, including when associated with other anesthetic drugs; for review, see [41]. These qualities made ketamine a rational choice for laboratory studies using rodents, mainly when associated with xylazine, a clonidine analog that, like other α_2-ARs agonists, presents analgesic, sedative, and muscle relaxant effects; for review, see [42]. The combination of both ketamine and xylazine results in prolonged anesthesia in rats [43]. Despite the lack of differences between basal blood pressure values, animals anesthetized with ketamine/xylazine presented a reduced basal heart rate than pentobarbital-anesthetized rats (Supplementary Table S1). This difference might be associated with the action of xylazine on α_2-ARs. However, it is essential to note that the pressor effect of clonidine found in ketamine/xylazine-anesthetized animals was significantly inhibited by yohimbine, an antagonist of α_2-ARs. Thus, although xylazine and clonidine share the same pharmacological target, we found no evidence that the interaction between these drugs and the α_2-ARs had any impact on the cardiovascular responses of the animals for the i.c.v.-administered clonidine.

It has already been suggested that the pressor effect of clonidine in conscious normotensive rats may result from its direct action on excitatory pathways in the anteroventral third ventricle and lateral hypothalamus [23]. Indeed, different studies have found hypertensive responses after clonidine microinjection in the hypothalamus, including the hypothalamic paraventricular nucleus [44,45]. It was suggested that clonidine acts in the paraventricular nucleus presynaptic α_2-ARs of GABAergic neurons, reducing the release of GABA neurotransmitters [43]. Thus, the excitatory activity of the paraventricular nucleus is increased by clonidine, generating hypertensive responses in conscious rats. Following this rationale, since pentobarbital directly modulates the opening of $GABA_A$ receptors, for review, see [26], the stimulatory activity of clonidine on the hypothalamic paraventricular nucleus is suppressed in pentobarbital-anesthetized rats, allowing the development of hypotension after the i.c.v. administration of clonidine. Interestingly, early studies demonstrated that the direct injection of clonidine in the anterior hypothalamic preoptic area of pentobarbital-anesthetized rats induced a fall in blood pressure and heart rate [46]. Since the ketamine/xylazine combination does not seem to interact with GABAergic pathways as does pentobarbital, it is reasonable to speculate that when administered by the i.c.v. route in ketamine/xylazine-anesthetized rats, the excitatory effect of clonidine on the hypothalamus overlaps the inhibitory effect generated by clonidine in the RVLM. Importantly, there is no information regarding cardiovascular effects caused by clonidine injection directly into the RVLM or the hypothalamus of ketamine/xylazine-anesthetized rats, and further investigations are necessary to confirm this hypothesis.

The ability of clonidine to reduce spontaneous GABAergic neurotransmission was previously demonstrated in the premotor cardioinhibitory vagal neurons [47], an effect also generated by dexmedetomidine [48] and entirely prevented by yohimbine [47,48]. The reduction in the cardiac frequency is a highly desired effect for drugs used against hypertension and may contribute to the clinical efficacy of clonidine in this condition. Interestingly, in our experiments, clonidine-induced bradycardia was much more intense in pentobarbital-anesthetized animals. The sum of the actions of pentobarbital and clonidine on the parasympathetic inhibitory innervation to the heart may explain this finding.

Clonidine binds to α_1-ARs with an α_2/α_1 binding ratio of 220:1 [49] and presents a similar affinity ratio between α_2 and I_1 imidazoline receptors in RVLM [50], which, once stimulated, also induce sympathoinhibitory responses [35]. To further characterize the ability of central α_2-ARs to induce pressor responses, we also administered dexmedetomidine and yohimbine in ketamine/xylazine-anesthetized rats. Dexmedetomidine is a more selective agonist of α_2-ARs, presenting an α_2/α_1 binding ratio of 1620:1 in rat brain membranes [49] and reduced interaction with imidazoline receptors; for review, see [51].

Yohimbine is an indole alkaloid that acts as a selective α_2AR antagonist and poorly interacts with imidazoline receptors [50]. As revealed in our experiments, the central administration of dexmedetomidine also induced sustained hypertensive responses in ketamine/xylazine-anesthetized rats, with even more pronounced bradycardia when compared with clonidine. Since the previous i.c.v. injection of yohimbine attenuated the pressor and bradycardic effects of both clonidine and dexmedetomidine, it is reasonable to conclude that α_2-ARs play an essential role in this cardiovascular regulatory pathway modulated by clonidine in ketamine/xylazine-anesthetized rats. However, because yohimbine did not abolish clonidine-induced pressor responses, the contribution of α_1 and imidazoline receptors, among others, cannot be ruled out.

Although we have aimed to compare the influence of ketamine/xylazine and pentobarbital anesthesia on clonidine effects, several other anesthetics have been widely used in experimental research, including but not limited to inhaled agents such as isoflurane. Considering our findings, it is reasonable to speculate that using anesthetics with different mechanisms of action can be a pharmacological strategy to further understand the effects of drugs on the brain. Notably, our results reinforce the need for increased attention and different approaches to explore the influence of anesthetic protocols on the action of cardiovascular drugs, mainly those that, like clonidine, are dependent on the central nervous system.

5. Limitations of This Study

The similar structure and pharmacological effects of xylazine and clonidine can be listed as a limitation of our study since the cardiovascular effects of centrally administered clonidine may have been influenced by xylazine in a way that we were not able to measure with our laboratory facilities. This study clearly showed how the anesthetic choice can affect the final effect of clonidine on the cardiovascular system but did not extend it to commonly used drugs such as inhalation anesthetics. Additionally, we also used only male animals in our experiments. Gender-related differences in the vascular effects of clonidine are scarce in the literature and limited to peripheral actions [52]. However, sex-related differences in the pharmacokinetics and pharmacodynamics of several cardiovascular drugs have been described [53]. Thus, the reproducibility of the anesthetic-dependent dual cardiovascular effects of clonidine must also be investigated in female rats, a missing point of our study.

6. Conclusions

The principal concept derived from the experiments reported in this study is that the final effect of centrally administered clonidine on both blood pressure and heart rate of rats is deeply influenced by the anesthetic combination, disclosing that anesthetic protocols adopted in earlier studies can have a biased influence in the rationale followed to understand the central effects of α_2-adrenergic receptor modulators. Indeed, our results disclose that in normotensive rats under ketamine/xylazine-induced anesthesia, the stimulation of α_2-ARs led to pressor and bradycardic responses, opposing the findings obtained in pentobarbital-anesthetized rats and resembling the findings in awake normotensive animals. An administration of these drugs in specific brain areas involved in blood pressure regulation can elucidate the mechanisms responsible for these differences. Importantly, our results reveal that despite the well-established antihypertensive action, clonidine and other α_2-adrenergic receptor agonists can promote hypertensive effects mediated by central mechanisms rather than by the activation of peripheral postsynaptic α1- or α_2-ARs. Our data reinforce the importance of revisiting the pharmacology of centrally acting agents for drug repurposing. Advances in this field can improve our knowledge regarding the central regulation of blood pressure, as well as the mechanisms of action and risks associated with the FDA-approved or off-label uses of clonidine in normotensive subjects from different populations.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/futurepharmacol4010003/s1. Supplementary Figure S1. Differential influence of pentobarbital- and ketamine/xylazine-induced anesthesia in the vascular effects of centrally administered clonidine in rats. Supplementary Table S1. Basal cardiovascular parameters in pentobarbital- and ketamine/xylazine-anesthetized rats. Supplementary Table S2. Effects of centrally administered clonidine in cardiovascular parameters of pentobarbital and ketamine/xylazine anesthetized rats. Supplementary Table S3. Influence of yohimbine in the cardiovascular effect of intracerebroventricular clonidine and dexmedetomidine in ketamine/xylazine anesthetized rats.

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