

RESEARCH ARTICLE

Effect of Isoflurane Anesthesia on the Heart Rate and Blood Pressure Response to Autonomic Nervous System Stimulation and Inhibition in Rats

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Objective: Many experimental studies aim to assess the autonomic nervous system (ANS), but this is often hampered by interactions with anesthetic drugs. We aimed to evaluate whether isoflurane anesthesia is suitable for ANS evaluation in rats. **Methods:** Six Wistar rats were anesthetized with isoflurane (4 L/min, 2.5%). Systolic blood pressure (SBP) and heart rate (HR) were measured at baseline and 20 min after sympathetic inhibition (propranolol, 5 mg/kg) and stimulation (isoproterenol, 2.5 mg/kg), and parasympathetic inhibition (atropine nitrate, 2 mg/kg) and stimulation (carbamylocholine, 0.4 mg/kg; acetylcholine, 0.1 mg/kg). Six additional rats were used to assess the effects of isoproterenol, carbamylocholine, and atropine nitrate in the absence of anesthesia. **Results:** Propranolol significantly decreased the HR and the SBP, whereas isoproterenol significantly increased the HR (all $p \leq 0.01$) in the isoflurane-anesthetized rats. However, the HR response to sympathetic stimulation was significantly reduced in the anesthetized compared to the non-anesthetized rats ($p = 0.03$). Carbamylocholine and acetylcholine significantly decreased both the HR and SBP (all $p < 0.05$) in the anesthetized rats, but the response to carbamylocholine administration was significantly more pronounced in the non-anesthetized rats ($p = 0.03$). Atropine nitrate significantly increased the HR ($p < 0.001$) in the non-anesthetized rats, but it had no effect on either the HR or the SBP in the presence of isoflurane anesthesia (both $p > 0.05$). **Conclusions:** Isoflurane anesthesia appears to interfere with both components of the ANS and is therefore not an optimal approach for experimental ANS evaluation. Our data indicate autonomic receptors and/or post-receptor mechanisms as the most likely site for isoflurane-ANS interactions.

Keywords: anesthesia, autonomic nervous system, blood pressure, heart rate, isoflurane

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Introduction

The sympathetic and the parasympathetic divisions of the autonomic nervous system (ANS) are designed to ensure rapid adaptation of whole-body functions to perturbations caused by a wide variety of internal and external factors. Outside of the central nervous system, the communication of the ANS with the effector organs is achieved through two-synapse pathways consisting of preganglionic and postganglionic neurons. The two synapses are located between the two neurons of the pathway, and between the postganglionic neuron and the effector organ, respectively [1]. The only exception to this rule is the adrenal medulla, which receives sympathetic inputs directly through the preganglionic fibers [1]. A disturbance in any component of these efferent pathways, including at synapse and/or post-synapse level, is therefore rapidly reflected into specific effector organs changes.

The cardiovascular system is one of the most relevant 'targets' of the ANS and sympatho-vagal imbalance is recognized as a key player in numerous cardiovascular disorders including heart failure, cardiac arrhythmias, and arterial hypertension [2-4]. Autonomic nervous system

evaluation in different clinical and experimental settings is therefore a major and constant concern in cardiovascular medicine. In experimental settings, full ANS evaluation often requires animal anesthesia. Unfortunately, since the vast majority of anesthetic drugs are known to interfere with the ANS at different levels [5], animal anesthesia often precludes adequate ANS evaluation.

Due to its effective myorelaxant and analgesic effects, ease of use, and rapid post-anesthesia recovery [6-8], isoflurane is one of the most commonly used anesthetic drugs in both clinical and experimental practice. Although previous studies have described possible interferences of isoflurane anesthesia with ANS functioning [9], isoflurane continues to be widely used in experimental studies aiming to evaluate the ANS [10].

To date, it remains unclear whether isoflurane anesthesia is suitable for experiments that target the evaluation of the sympathetic and parasympathetic nervous systems. Therefore, we aimed to evaluate the effects of isoflurane anesthesia on heart rate (HR) and systolic blood pressure (SBP) changes in response to sympathetic and parasympathetic stimulation and inhibition in rats.

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Methods

Studied animals

The study was performed on 12 adult male Wistar rats obtained from the Experimental Animal Center of the university. All animals were housed in a climate-controlled room (21°C to 23°C), with a 12-h light/12-h dark cycle, in an accredited animal facility. The rats were housed individually in polycarbonate cages and had free access to water and standard food throughout the study. All protocols were performed in accordance with the International Council for Laboratory Animal Science guidelines (Directive 2010/63/EU) and were approved by the local Ethics Committee.

Assessment of heart rate and systolic blood pressure changes in response to sympathetic and parasympathetic stimulation and inhibition

Heart rate and SBP changes in response to sympathetic and parasympathetic stimulation and inhibition were assessed under isoflurane anesthesia in 6 of the 12 rats included in the study. All 6 rats were anesthetized using a mixture of isoflurane (2.5%) and oxygen (4 L/min) and underwent continuous ECG monitoring throughout the experimental procedures, as described previously [11].

Sympathetic and parasympathetic agonists and antagonists were injected subcutaneously in all 6 animals. The rats received a single sympathetic/parasympathetic agonist/antagonist per day. In order to avoid interferences from circadian variations in ANS functioning, all injections were performed at the same hour. Sympathetic stimulation was achieved using isoproterenol (2.5 mg/kg), whereas sympathetic inhibition was achieved using propranolol (5.0 mg/kg). Parasympathetic stimulation was performed using both acetylcholine (0.1 mg/kg) and carbamylcholine (0.4 mg/kg), a cholinergic agonist resistant to degradation by cholinesterases. Atropine nitrate (2.0 mg/kg), a muscarinic receptor antagonist that does not cross the blood-brain barrier, was used for parasympathetic inhibition.

Heart rate and SBP were measured for each animal at baseline and 20 min after each drug administration. The SBP was measured using the photoplethysmographic method, as described previously [11]. Heart rate values were derived from the surface ECG recordings. All data were analyzed using a program developed in our laboratory using the LabVIEW 2010 software (National Instruments, Austin, TX). Heart rate and SBP values obtained 20 min following drug administration were compared with the baseline values for each animal and for each administered drug.

The 6 remaining rats were implanted with radiotelemetry ECG transmitters, as described previously [11]. After one week of post-implantation recovery, HR changes in response to atropine nitrate (2.0 mg/kg), isoproterenol (2.5 mg/kg), and carbamylcholine (0.4 mg/kg) were assessed in the absence of anesthesia (i.e., in freely moving, 'conscious' rats). Due to technical reasons, SBP could not be measured in these 6 non-anesthetized rats.

Statistical analyses

Statistical analyses were performed using the GraphPad InStat 3 Software (San Diego, CA). All data are expressed as means \pm standard error of the mean. Baseline HR and SBP values were compared with post-drug administration values using the paired Student *t*-test or the Wilcoxon matched-pairs signed-ranks test, as appropriate. The baseline HR and the magnitude of the HR response to isoproterenol and carbamylcholine administration were compared between the anesthetized and the non-anesthetized rats using the unpaired Student *t*-test or the Mann Whitney *U* test, as appropriate. A *p*-value < 0.05 was considered statistically significant.

Results

Heart rate values at baseline (i.e., prior to any drug administration) were significantly higher in the isoflurane-anesthetized compared to the non-anesthetized rats (364.4 ± 9.2 bpm *versus* 294.1 ± 14.4 bpm; $p < 0.01$).

Heart rate and systolic blood pressure changes in response to sympathetic stimulation and inhibition in isoflurane-anesthetized and non-anesthetized rats

As expected, sympathetic stimulation with isoproterenol significantly increased the HR compared to baseline in both the isoflurane-anesthetized ($p < 0.01$; Table I) and the non-anesthetized (390.2 ± 10.8 bpm *versus* 266.6 ± 5.7 bpm; $p < 0.0001$) rats. However, the HR response to isoproterenol administration was significantly higher in the non-anesthetized compared to the anesthetized rats ($+123.6 \pm 9.9$ bpm *versus* $+72.5 \pm 14.4$ bpm; $p = 0.03$). Isoproterenol-induced peripheral vasoconstriction precluded SBP measurement following drug administration in all rats. In the anesthetized rats, sympathetic inhibition using propranolol significantly decreased both the HR ($p = 0.01$) and the SBP ($p < 0.001$) compared to the baseline values (Table I).

Heart rate and systolic blood pressure changes in response to parasympathetic stimulation and inhibition in isoflurane-anesthetized and non-anesthetized rats

In the isoflurane-anesthetized rats, parasympathetic stimulation with both acetylcholine and carbamylcholine led to a significant decrease in both the HR and the SBP compared to the baseline values (all $p < 0.05$; Table I). Similarly, carbamylcholine administration also led to a significant decrease in the HR in the non-anesthetized rats (294.1 ± 14.4 bpm *versus* 139.9 ± 7.6 bpm; $p < 0.001$). However, the HR response to carbamylcholine administration was significantly less pronounced in the anesthetized compared to the non-anesthetized rats (-23.2 ± 6.5 bpm *versus* -154.2 ± 18.2 bpm; $p = 0.03$). In the anesthetized rats, HR changes (-43.7 ± 6.3 bpm for acetylcholine *versus* -23.2 ± 6.5 bpm for carbamylcholine; $p = 0.06$) and SBP changes (-20.9 ± 1.5 mmHg for acetylcholine *versus* -8.0 ± 3.2 mmHg for carbamylcholine; $p = 0.06$) were not significantly different

Table I. Heart rate and systolic blood pressure in isoflurane-anesthetized rats at baseline and 20 minutes after sympathetic stimulation / inhibition (using isoproterenol, 2.5 mg/kg / propranolol, 5.0 mg/kg) and parasympathetic stimulation / inhibition (using acetylcholine, 0.1 mg/kg, and carbamylcholine, 0.4 mg/kg / atropine nitrate, 2.0 mg/kg)

	Baseline (n = 6)	20 min after drug administration (n = 6)	p-value
Sympathetic stimulation			
Isoproterenol			
HR (bpm)	364.6 ± 7.8	437.1 ± 11.3	< 0.01
SBP (mmHg)	112.5 ± 4.4	-	-
Sympathetic inhibition			
Propranolol			
HR (bpm)	349.8 ± 10.6	252.5 ± 4.5	0.01
SBP (mmHg)	113.3 ± 4.5	93.3 ± 3.3	< 0.001
Parasympathetic stimulation			
Acetylcholine			
HR (bpm)	369.8 ± 5.0	326.1 ± 3.2	< 0.01
SBP (mmHg)	112.5 ± 2.1	91.6 ± 2.7	< 0.0001
Carbamylcholine			
HR (bpm)	364.4 ± 9.2	341.20 ± 7.5	< 0.01
SBP (mmHg)	116.0 ± 5.8	108.0 ± 3.8	0.03
Parasympathetic inhibition			
Atropine nitrate			
HR (bpm)	344.6 ± 13.5	336.1 ± 7.2	0.35
SBP (mmHg)	107.5 ± 12.9	105.8 ± 15.7	0.87

Because of the strong peripheral vasoconstriction, systolic blood pressure (SBP) could not be measured after isoproterenol administration. Data are expressed as means ± standard error of the mean. P-values were obtained using the paired Student t-test or the Wilcoxon matched-pairs signed-ranks test, as appropriate. HR – heart rate.

following administration of equivalent doses of acetylcholine and carbamylcholine, despite the different sensitivity of the two drugs to degradation by cholinesterases.

Importantly, atropine nitrate administration did not induce significant HR or SBP changes (both $p > 0.05$; Table I) in the isoflurane-anesthetized rats. In contrast, in the absence of anesthesia, atropine nitrate caused a significant increase in HR compared to the baseline values (420.8 ± 9.5 bpm *versus* 305.0 ± 17.2 bpm; $p < 0.001$).

Discussion

The main findings of the present study were that: (1) HR and SBP responses to sympathetic stimulation and inhibition remained active in the presence of isoflurane anesthesia, although (2) isoflurane blunted the HR response to sympathetic stimulation; (3) HR and SBP responses to parasympathetic stimulation remained active in the presence of isoflurane anesthesia, and (4) were similar regardless if the parasympathetic agonist was sensitive or non-sensitive to degradation by cholinesterases; however, (5) the HR response to parasympathetic stimulation was also blunted by isoflurane anesthesia; moreover, (6) isoflurane anesthesia canceled completely the HR and SBP responses to parasympathetic inhibition, whereas (7) in the absence of isoflurane anesthesia, parasympathetic inhibition exhibited its typical tachycardia effect.

Previous studies have shown that isoflurane interferes with ANS activity [9,12]. However, a significant number of studies have failed to demonstrate whether such interferences truly exist, whether they affect both or only one of the two ANS divisions, and, if they do exist, at what level they occur [6,12-14]. The present study demonstrates that HR and SBP changes in response to sympathetic stimulation and inhibition with isoproterenol and propranolol,

respectively, are not radically altered under isoflurane anesthesia. However, the response to sympathetic stimulation was significantly reduced in the presence of isoflurane anesthesia, suggesting that isoflurane exhibits antiadrenergic effects. Although some studies have failed to confirm this hypothesis, and have even associated increased isoflurane concentrations with an increase in HR and SBP values [6], other studies have indeed suggested that isoflurane may exert sympatholytic effects, leading to hypotension [13], decreased sympathetic nerve activity [13], depressed baroreceptor reflex control of the HR [15], and decreased plasma norepinephrine levels [16]. Further studies will have to identify the exact location of this isoflurane-sympathetic nervous system interaction. However, the fact that in the present study isoflurane anesthesia blunted the response of the HR to direct sympathetic receptor stimulation suggests that isoflurane-sympathetic nervous system interferences are likely to occur at receptor and/or post-receptor level.

The results of the present study also demonstrate that isoflurane anesthesia does not cancel completely the cardiovascular response to parasympathetic stimulation induced by acetylcholine and carbamylcholine. However, the HR response to direct cholinergic receptors stimulation was also significantly reduced in the presence of isoflurane anesthesia, suggesting that isoflurane may exhibit not only antiadrenergic, but also anticholinergic effects. This anticholinergic effect of isoflurane is also supported by the higher baseline HR recorded in the anesthetized compared with the non-anesthetized rats. Moreover, in the present study, isoflurane anesthesia completely cancelled the HR and SBP responses to atropine nitrate administration, further supporting the hypothesis of a strong isoflurane-induced cholinergic inhibition. Indeed, in isoflurane-anesthetized children, atropine administration also failed to in-

crease the SBP, although it was associated with an increase in the HR [17].

Theoretically, this isoflurane-parasympathetic nervous system interaction may occur at any anatomical level (i.e., at the level of the autonomic centers, of the pre- and/or post-ganglionic neurons or of the parasympathetic ganglia, at receptor and/or post-receptor level). In the present study, HR and SBP changes induced by acetylcholine and carbamylcholine were not significantly different in the anesthetized rats, suggesting that isoflurane is unlikely to have interfered with cholinesterase activity. However, the statistical power of this analysis could have been affected by the relatively small number of animals used in this study. An interference of isoflurane with cholinesterase activity cannot be therefore excluded based solely on our data. Given that isoflurane anesthesia completely blunted the cardiovascular response to atropine nitrate, a drug that does not cross the blood-brain barrier, the interference of isoflurane with the parasympathetic nervous system is also unlikely to have involved the autonomic centers. Although interactions at preganglionic, ganglionic, or postganglionic level, and isoflurane-induced decrease in acetylcholine synthesis, as suggested by Su et al. [18], cannot be excluded, the altered response to direct cholinergic receptors stimulation coupled with the complete lack of response to cholinergic receptor blockade observed in the present study suggests that this isoflurane-parasympathetic nervous system interference is most likely to occur at receptor and/or post-receptor level. This hypothesis is also supported by a number of previous studies, which have related isoflurane anesthesia with protein kinase C-mediated muscarinic receptors inhibition [15], as well as with reduced activation rate of muscarinic receptors-activated K^+ channels [16].

The present study shows that in rats, the cardiovascular response to sympathetic stimulation/inhibition remains present under isoflurane anesthesia. However, our results indicate that this response may be significantly lower than that recorded in the conscious state. More importantly, the present study suggests that isoflurane anesthesia may cancel completely the cardiovascular response to cholinergic inhibition, making atropine usage in the setting of isoflurane anesthesia rather futile. One should also be aware that, given its obvious interactions with both ANS branches, isoflurane anesthesia may be associated with intraoperative hemodynamic abnormalities. If also applicable to humans, these results could have major clinical importance. Further studies will have to confirm these findings in clinical settings. From an experimental point of view, the results of the present study suggest that isoflurane anesthesia is not appropriate for ANS evaluation.

Potential limitations

The main limitation of the present study is related to the relatively small number of animals, which could have affected the statistical power of the study. Systolic blood pressure measurement in the non-anesthetized rats could have

brought important additional data. Unfortunately, due to technical reasons, measurement of SBP was not feasible in the non-anesthetized rats in the present study. Although the present data indicate autonomic receptors and/or post-receptor mechanisms as the most likely site for isoflurane-ANS interactions, future studies will have to establish the exact location of these interactions and to elucidate the molecular mechanisms responsible for isoflurane-ANS interferences.

Conclusion

The present study demonstrates that isoflurane anesthesia exerts both antiadrenergic and anticholinergic effects, although it does not cancel completely the cardiovascular response to sympathetic and parasympathetic stimulation. However, isoflurane appears to completely abolish the cardiovascular response to cholinergic receptors blockade. Cholinergic receptors and/or post-receptor mechanisms appear to be the most likely site for this isoflurane-parasympathetic nervous system interaction. These data suggest that isoflurane anesthesia may affect patients' intraoperative hemodynamic status and their response to therapeutic ANS manipulation, and demonstrate that isoflurane is not an optimal approach for experimental ANS evaluation.

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Authors' contribution

BAI (Data curation; Formal analysis; Investigation; Methodology; Writing – original draft); GT (Investigation; Methodology; Writing – review & editing); MC (Data curation; Formal analysis; Investigation; Methodology; Writing – review & editing); GA (Investigation; Methodology; Writing – review & editing); HVB (Investigation; Methodology; Funding acquisition; Writing – review & editing); ŞRC (Conceptualization; Supervision; Validation; Writing – review & editing); SA (Conceptualization; Formal analysis; Project administration; Resources; Supervision; Validation; Writing – original draft; Writing – review & editing). The first two authors contributed equally to this paper and both should be viewed as first authors.

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