Isoflurane Depresses Baroreflex Control of Heart Rate in Decerebrate Rats

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Background: Isoflurane inhibits baroreflex control of heart rate (HR) by poorly understood mechanisms. The authors examined whether suprapontine central nervous system cardiovascular regulatory sites are required for anesthetic depression.

Methods: The effects of isoflurane (1 and 2 rat minimum alveolar concentration [MAC]) on the baroreflex control of HR were determined in sham intact and midcollicular-transected decerebrate rats. Intravenous phenylephrine (0.2–12 μg/kg) and nitroprusside (1–60 μg/kg) were used to measure HR responses to peak changes in mean arterial pressure (MAP). Sigmoidal logistic curve fits to HR–MAP data assessed baroreflex sensitivity (HR/MAP), HR range, lower and upper HR plateau, and MAP at half the HR range (BP_{50}). Four groups (two brain intact and two decerebrate) were studied before, during, and after isoflurane. To assess sympathetic and vagal contributions to HR baroreflex, β-adrenoceptor (1 mg/kg atenolol) or muscarinic (0.5 mg/kg methyl atropine) antagonists were administered systemically.

Results: Decerebration did not alter resting MAP and HR or baroreflex parameters. Isoflurane depressed baroreflex slope and HR range in brain-intact and decerebrate rats. In both groups, 1 MAC reduced HR range by depressing peak reflex tachycardia. Maximal reflex bradycardia during increases in blood pressure was relatively preserved. Atenolol during 1 MAC did not alter maximum reflex tachycardia. In contrast, atropine during 1 MAC fully blocked reflex bradycardia. Therefore, 1 MAC predominantly depresses sympathetic components of HR baroreflex. Isoflurane at 2 MAC depressed both HR plateaus and decreased BP_{50} in both groups.

Conclusions: Isoflurane depresses HR baroreflex control by actions that do not require suprapontine central nervous system sites. Isoflurane actions seem to inhibit HR baroreflex primarily by the sympathetic nervous system.

VOLATILE anesthetics, such as isoflurane, disrupt regulation of the circulation,[1] although their mechanisms of action and targets are poorly understood. These anesthetics can impair cardiovascular function by acting directly on peripheral organs, such as the heart[2,3] and blood vessels,[4,5] as well as by compromising central nervous system (CNS) targets, including baroreceptor reflex (BRX) neurons, and these various actions together result in altered cardiovascular regulation.[6,7] One of the basic autonomic regulatory reflexes is the cardiac BRX. The BRX control of heart rate (HR) constitutes a classic negative feedback system in which afferent sensory information from arterial baroreceptors enters the medulla at the nucleus tractus solitarius (NTS).[8] These NTS neurons in turn activate brain stem cardioinhibitory vagal neurons in nucleus ambiguus and dorsal motor nucleus as well as inhibit sympathoexcitatory neurons in the ventrolateral medulla. Although supramedullary brain structures can importantly modulate BRX performance, all of the required neural circuitry for this basic BRX is present in the medulla and the spinal cord.[9]

In humans and animals, it is well-established that isoflurane depresses BRX control of HR.[7,10,11] Isoflurane likely affects all components of HR-BRX (afferent, CNS, efferent, and end organs).[7] However, bulbar and suprapontine contributions to BRX depression by isoflurane are uncertain. Infracollicular decerebration has no effect on BRXs for some anesthetics (*e.g.*, ketamine), whereas other agents (*e.g.*, althesin) depressed the HR-BRX to a greater degree after decerebration.[12] In addition, volatile anesthetics, including halothane, may differentially depress brain stem cardiovascular centers compared with supramedullary regions.[13]

Therefore, suprapontine modulation of medullary cardiovascular centers likely varies among anesthetics and across also CNS sites. It is not known whether suprapontine CNS regulatory sites (*e.g.*, hypothalamus, thalamus, cortex) are required for HR-BRX depression by isoflurane. In addition, many previous experiments examined cardiovascular control mechanisms during basal anesthesia.[6,14] Basal anesthetics have the potential to confound interpretation because some alter additional physiologic functions, including the baroreceptor control mechanism.[13,15]

Therefore, the purpose of the current study was to test whether actions at suprapontine brain regions were necessary for isoflurane to depress the HR-BRX. We compared the effects of isoflurane on BRX control of HR in sham-intact and midcollicular-transected decerebrate rats. In addition, we assessed the contributions of sympathetic and parasympathetic pathways on HR-BRX depression by isoflurane. We found that isoflurane depresses BRX control of HR in brain-intact and decerebrate rats to a similar degree, and isoflurane depresses the HR-BRX predominantly by inhibiting the sympathetic component.

Methods

Chronic Instrumentation

All animal procedures and protocols were approved by the Animal Care and Use Committee of the Oregon

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Health & Science University (Portland, Oregon) and were designed in accordance with the recommendations of the Panel on Euthanasia of the American Veterinary Medical Association and the National Institutes of Health publication *Guide for the Care and Use of Laboratory Animals*. The detailed methods for chronic cardiovascular instrumentation were described previously.[16] Briefly, male Sprague-Dawley rats weighing 350–400 g (Harlan, Indianapolis, IN) were anesthetized with isoflurane, and polyethylene catheters were placed in one femoral artery (polyethylene 50) for arterial pressure measurement and in both femoral veins (polyethylene 10) for drug administration, one for pressor (phenylephrine) and the other for depressor (sodium nitroprusside) drug administration. These catheters were filled with heparinized saline, led subcutaneously to the scruff of the neck, and externalized through a small incision. Three stainless wire electrodes were placed under the skin of left and right front legs and left hind leg for electrocardiographic monitoring. Rats were treated prophylactically with amoxicillin (150 mg/kg intramuscularly) and were allowed to recover for 2 days.

Monitoring Hemodynamic Parameters

On the day of the experiment, each rat was placed in a rat restrainer, and the arterial catheter was connected to a pressure transducer (Baxter, Round Lake, IL) to monitor blood pressure (BP). The BP signal was digitally sampled by a data acquisition system (PowerLab/4s; AD Instruments, Grand Junction, CO) and was displayed on a microcomputer (Dell, Austin, TX). HR was derived from the BP signal. Lead II electrocardiographic signals and other signals were also continuously recorded on a pen recorder (Grass Instrument 78, Quincy, MA). Studies were performed in a quiet room.

Baroreceptor Reflex Studies

A series of graded intravenous bolus injections of phenylephrine (0.2–12 μg/kg; 1–60 μl of a 200-μg · kg^{-1} · ml^{-1} solution) and nitroprusside (1–60 μg/kg; 1–60 μl of a 1-mg · kg^{-1} · ml^{-1} solution) were used to obtain HR responses to peak mean arterial pressure (MAP) changes in an alternating order of delivery.[17] Sufficient time was allowed for both MAP and HR to return to resting values between injections. Generally, seven increases and seven decreases in BP were obtained in each condition. Resting values for MAP and HR between vasoactive drug tests were measured for 30 s before each test, and these values (MAP and HR before 7 phenylephrine and 7 nitroprusside tests) were averaged to represent the resting pretest conditions (resting MAP and resting HR). Therefore, they may not represent true steady-state resting MAP and HR before and after isoflurane (*e.g.*, after autonomic antagonist). MAP–HR data (resting and evoked reflex responses) in individual rats were fitted to a sigmoidal logistic equation (Boltzmann

equation, Origin 6.1; Origin Labs, Northampton, MA) as follows:

$$HR = A_1 - A_2/[1 + e^{(X-Xo)dx}] + A_2,$$

where A_1 = upper HR plateau, A_2 = lower HR plateau, $A_1 - A_2$ = HR range, X = MAP, Xo = BP$_{50}$ (MAP at half the HR range), and dx = a curvature coefficient that is independent of range. The average gain (G) or slope of the curve was determined by

$$G = -(A_1 - A_2)/dx \times 4.56.$$

All individual curves in each rat were restrained to go through the average resting MAP and HR values before phenylephrine or nitroprusside injections. The goodness of fit of the individual curves was determined by chi-square and R^2. Parameters from individual curves (resting MAP and HR, slope, dx, A_1, A_2, and BP$_{50}$) were averaged, and then average curves were reconstructed for each group of rats.[17]

Decerebration

Decerebration was performed at midcollicular level according to methods described by Faber *et al.*[18] After testing the HR-BRX in the awake state, the same rats were anesthetized with isoflurane (2–2.5% in oxygen), intubated and fixed in a standard stereotaxic instrument (Cartesian Research, Inc., Sandy, OR). A midline incision exposed the skull, and the skin was retracted to the sides. Two bilateral craniectomy furrows (5 × 3 mm) were made approximately 6.5 mm posterior to the bregma with a No. 8 dental burr drill. The dura was removed, taking care not to injure the superior sagittal sinus. Using a micromanipulator with a blunt, hooked knife blade, the entire brain stem was transected at midcollicular level (6.5 mm posterior to the bregma according to the Paxinos and Watson atlas).[19] This procedure spares the mid-sagittal venous sinus and large blood vessels at the base of the midbrain. Sham operations were performed identically with the exception of the decerebration knife cut. Blood loss was minimal (averaging less than 0.5 ml). The skull was closed with bone wax before proceeding with the experimental protocols. After the experiment, the brain was removed, fixed overnight in a 10% buffered formalin solution, and examined to verify the completeness of the brain stem transection.

Experimental Protocol

Animals were divided into four groups (n = 6 in each group). Two groups served as controls (sham), and two groups were decerebrated. Each group received 1 or 2 minimum alveolar concentration (MAC) isoflurane (rat, 1.45 and 2.9%).[20] BRX tests were performed in each group before, during, and after isoflurane exposure. For maintained anesthesia level, rats were initially anesthe-

tized with isoflurane in a closed chamber followed by intubation with a 16-gauge intravenous catheter (Critikon, Tampa, FL). During surgeries, anesthesia was maintained with isoflurane (2–2.5% in oxygen) using a Fortec Vaporizer (Cyprane, Keighley Yorkshire, United Kingdom). End-tidal isoflurane concentration and carbon dioxide were continuously monitored with an Ohmeda 6000 Multi Gas Analyzer (Ohmeda, Madison, WI) through a fine sampling tube placed in the wide-bore base of the endotracheal tube. Respiration was controlled by a ventilator to achieve end-tidal carbon dioxide of 32–33 mmHg. Body temperature was maintained at 36.5–37.5°C by a temperature-controlled heating pad and lamp.

After initial conscious intact measurements, including the BRX tests, rats were anesthetized, and either a decerebration or a sham operation was then performed. When these preparations were completed, isoflurane was adjusted to achieve an end-tidal concentration of 1 or 2 MAC (rat, 1.45 or 2.9%).[20] When a desired isoflurane concentration was reached, at least another 30 min of equilibration time was allowed before BRX testing. When these measurements were completed, anesthesia was halted, and the animals were allowed to recover for 2 h (zero isoflurane). Rats were extubated, atmospheric oxygen was supplemented near the face, and the BRX test was repeated in the absence of isoflurane. These recovery, zero anesthesia data for brain-intact (sham-treated) and decerebrate rats were used in comparisons as controls.

In a separate group of decerebrate and sham-treated animals, sympathetic and vagal contributions to HR-BRX in each condition were assessed. After testing the BRX at 1 MAC isoflurane, peripherally acting autonomic antagonists were administered intravenously. The β-adrenoceptor antagonist atenolol at 1 mg/kg (20 μl of a 50-mg · kg^{-1} · ml^{-1} solution) or the muscarinic antagonist methyl atropine at 0.5 mg/kg (20 μl of 25-mg · kg^{-1} · ml^{-1} solution) was administered. Ten minutes later, the BRX test was repeated in the presence of isoflurane and antagonist. The appropriate effective doses of antagonists were previously determined.[16]

Arterial Blood Gases, Hematocrit, Plasma Sodium, and Potassium

To determine whether alterations in acid–base balance, electrolytes, or hematocrit could have contributed to the observed changes, arterial blood samples (0.5 ml) were drawn immediately before BRX tests in three conditions (conscious brain-intact state, during isoflurane administration after decerebration or sham operation, and zero isoflurane state).

Statistics

All data are expressed as mean ± SD. MAP–HR BRX curves were obtained in each condition (before inter-

ventions, during isoflurane, and without anesthetic, *i.e.*, zero isoflurane) in each animal. The hemodynamic values and parameters of the curve fits (resting MAP and HR, slope, dx, A_1, A_2, BP_{50}) were averaged. Comparisons among mean values of the parameters were made within and between groups using repeated measures of two-way analysis of variance. For significant interactions, the Scheffé F test was used for *post hoc* comparisons (Statview®, 2nd edition, 1998; SAS Institute Inc., Cary, NC). Comparisons of the parameters of BRX curves in the presence and absence of each antagonist during isoflurane exposure among groups were analyzed by two-way analysis of variance followed by Scheffé F test because the zero isoflurane BRX curves were pooled from different animals. *P* values of less than 0.05 were considered significant. Group summary curves (n = 6) were constructed from the averaged curve parameters. The quality of the curve fits, R^2 as assessed by the chi-square values for the logistic regression, was excellent. For intact animals, these R^2 values averaged 0.961 ± 0.305 and 0.981 ± 0.007 for conscious awake states (before 1 and 2 MAC isoflurane), 0.835 ± 0.221 during 1 MAC isoflurane, and 0.617 ± 0.203 during 2 MAC isoflurane. For sham-treated 1 and 2 MAC intact controls (zero isoflurane), R^2 averaged 0.955 ± 0.038 and 0.964 ± 0.368. For the respective decerebrate animals, these R^2 averaged 0.973 ± 0.015 and 0.970 ± 0.016 for conscious awake state (before 1 and 2 MAC isoflurane), 0.916 ± 0.05 during 1 MAC isoflurane, and 0.602 ± 0.250 during 2 MAC isoflurane. For the respective decerebrate controls, R^2 averaged 0.910 ± 0.021 and 0.956 ± 0.026 for decerebrate 1 and 2 MAC controls (zero isoflurane).

Drugs

Isoflurane was purchased from Abbott Laboratories (North Chicago, IL). Phenylephrine hydrochloride, sodium nitroprusside, methyl atropine bromide, and atenolol were purchased from Sigma Chemical Company (St. Louis, MO). All drugs were prepared in saline before each experiment.

Results

Hemodynamics in Brain-intact and Decerebrate Rats

Because preliminary experiments showed that 2 h was sufficient for intact animals to fully recover from anesthetic effects with respect to BRX sensitivity, this time interval was used throughout as the respective control zero-anesthetic period after decerebration and sham operations. Basal resting MAP and HR before isoflurane introduction were similar among groups (table 1). Decerebration (zero isoflurane) did not alter resting MAP and HR. Isoflurane effects on resting MAP and HR were similar in sham-intact and decerebrate rats. Isoflurane at

Table 1. Logistic Baroreflex Parameters in Intact and Decerebrate Rats with 1 and 2 MAC Isoflurane

Experimental Group		Brain-stem Intact			Decerebrate		
		Conscious Intact	Isoflurane	Zero Isoflurane (Sham)	Conscious Intact	Isoflurane	Zero Isoflurane (Decerebrate)
1 MAC	Basal MAP (mmHg)	122 ± 5	112 ± 4*†	129 ± 8	120 ± 6	103 ± 9*†	124 ± 7
	Basal HR (beats/min)	357 ± 22	334 ± 31	351 ± 14	357 ± 24	327 ± 26	338 ± 39
	A1 (beats/min)	476 ± 16	369 ± 33*†	502 ± 17	482 ± 23	350 ± 26*†	448 ± 50
	A2 (beats/min)	232 ± 27	244 ± 37	221 ± 32	189 ± 42	231 ± 23	187 ± 58
	HR range (beats/min)	244 ± 16	125 ± 36*†	281 ± 30	293 ± 29	119 ± 15*†	260 ± 26
	BP50 (mmHg)	122 ± 7	120 ± 14	126 ± 13	127 ± 10	119 ± 6	125 ± 15
	Slope (beats · min^{-1} · mmHg^{-1})	−3.4 ± 0.4	−2.1 ± 0.6*†	−3.2 ± 0.7	−3.5 ± 0.7	−2.2 ± 0.3*†	−3.6 ± 0.6
2 MAC	Basal MAP (mmHg)	124 ± 10	84 ± 8*†‡	130 ± 7	121 ± 2	73 ± 3*†‡	121 ± 6
	Basal HR (beats/min)	358 ± 14	344 ± 16	337 ± 19	346 ± 21	328 ± 27	334 ± 11
	A1 (beats/min)	488 ± 37	359 ± 19*†	472 ± 42	479 ± 40	347 ± 28*†	459 ± 43
	A2 (beats/min)	235 ± 33	308 ± 19*†‡	198 ± 20	192 ± 56	305 ± 26*†‡	178 ± 14
	HR range (beats/min)	253 ± 35	51 ± 8*†‡	273 ± 40	287 ± 83	42 ± 12*†‡	281 ± 48
	BP50 (mmHg)	120 ± 8	97 ± 10*†‡	131 ± 12	124 ± 3	75 ± 19*†‡	122 ± 5
	Slope (beats · min^{-1} · mmHg^{-1})	−3.1 ± 0.3	−0.8 ± 0.3*†‡	−3.2 ± 0.3	−3.1 ± 0.7	−0.6 ± 0.3*†‡	−2.9 ± 0.6

Values are mean ± SD. N = 6 in each group.

* Significant difference from zero isoflurane ($P < 0.05$).   † Significant difference between isoflurane and conscious intact ($P < 0.05$).   ‡ Significant difference between respective 1 and 2 minimum alveolar concentration (MAC; $P < 0.05$).

MAP = mean arterial pressure; HR = heart rate; A1 = upper HR plateau; A2 = lower HR plateau; HR range = difference between upper and lower HR plateau; BP50 = MAP at half HR range.

1 and 2 MAC decreased basal MAP in a dose-dependent manner in sham as well as in decerebrate rats. The extent of the BP decreases was similar whether the brain was intact or decerebrate. In contrast, isoflurane at 1 and 2 MAC did not alter the resting HR (table 1).

Effects of Isoflurane on Heart Rate Baroreceptor Reflex in Brain-intact and Decerebrate Rats

Decerebration (zero isoflurane) did not alter BRX function parameters (slope, HR range, A_1, A_2, and BP_{50}) compared with conscious brain-intact states or sham-treated rats (fig. 1, table 1). Isoflurane depressed BRX sensitivity and HR range in a concentration–dependent manner in sham-intact and decerebrate rats (table 1, fig. 1). Isoflurane-induced depression of the BRX was similar between the two. At 1 MAC, isoflurane reduced the BRX-HR range predominantly depressing the upper (A_1) and sparing the lower HR plateau (A_2) (table 1, fig. 1). At this isoflurane level, decreasing the BP with nitroprusside evoked little increase in HR, whereas phenylephrine-induced increases

Fig. 1. Effects of isoflurane (ISO) on mean arterial pressure (MAP)–heart rate (HR) baroreflex curves for brain-intact/sham (*A* and *C*) and decerebrate (Dec; *B* and *D*) rats. Each panel shows curves for the following conditions: (1) before isoflurane (conscious intact); (2) during isoflurane (1 or 2 minimum alveolar concentration [MAC]) exposure after sham or decerebration; and (3) after recovery from isoflurane (zero isoflurane). Note that even 1 MAC isoflurane depressed the tachycardia portion of the curves to a greater extent than the bradycardia portion. Curves represent average baroreflex curve for six animals. Closed circles indicate resting level of MAP and HR for each curve. bpm = beats/min.
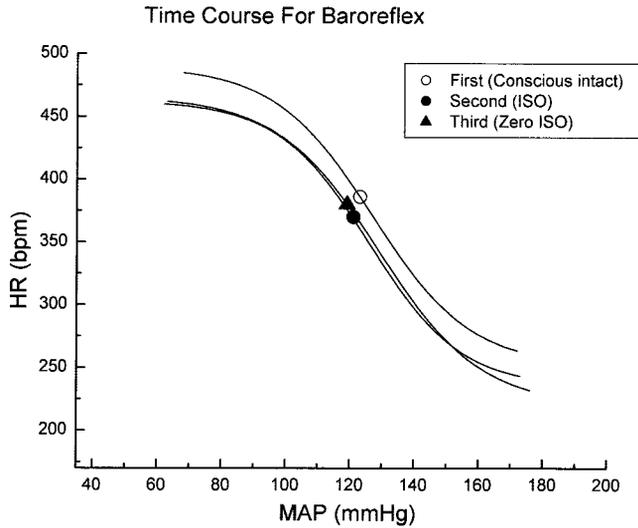
Time Course For Baroreflex
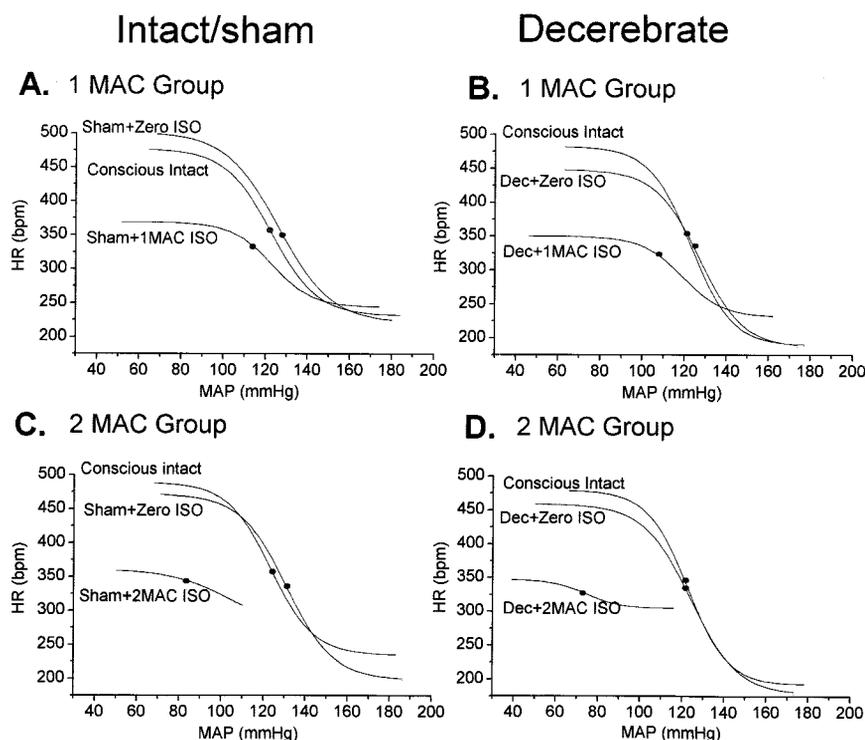
Fig. 2. Stability of mean arterial pressure (MAP)–heart rate (HR) baroreflex curves in conscious brain stem–intact rats. Three time-matched control baroreceptor reflex curves correspond to the three protocol intervals. The initial period (First) is for conscious intact controls (before isoflurane [ISO]). Second corresponds to isoflurane exposure after sham or decerebration operation. Third corresponds to the recovery period from isoflurane exposure (Zero ISO). Note that no differences were detected across the time frame of the full protocol, indicating a stable and highly reproducible baroreceptor reflex. Each trace represents the average baroreflex curve for three animals. Resting levels of MAP and HR for each curve are indicated by the large symbols corresponding to each period. bpm = beats/min.

in BP elicited substantial decreases in HR that were similar to controls. However, at 2 MAC, isoflurane depressed upper and lower HR plateaus (table 1, fig. 1) and shifted BP_{50} to lower pressures. Time-matched controls (sham) in a

limited number of animals showed that MAP, HR, and BRX parameters were remarkably stable with time, indicating high reproducibility with these methods (fig. 2). Arterial blood gas (pHa, arterial carbon dioxide tension [Pa_{CO_2}], arterial oxygen tension [Pa_{O_2}]), electrolytes (Na^+ and K^+), and hematocrits were similar between the brain-intact and decerebrate rats in all experimental settings (during brain-intact, isoflurane exposure, and zero anesthetic periods). However, Pa_{O_2} was higher (468–515 mmHg) during isoflurane administration compared with other time periods because supplemental oxygen was administered with isoflurane.

Effects of Isoflurane on Heart Rate Baroreceptor Reflex in the Presence of Autonomic Antagonists

To assess sympathetic and parasympathetic contributions to the changes in BRX characteristics with decerebration or with isoflurane, the BRX tests were performed in the presence and absence of atenolol or methyl atropine. Blockade of the sympathetic contribution to the HR-BRX with atenolol had little additional effect on the 1 MAC isoflurane depression observed in the brain-intact group (sham) (fig. 3, table 2). The upper (A_1) and lower (A_2) HR plateaus were similar with and without atenolol during isoflurane, although A_1 was significantly lower in both compared with the awake, zero anesthetic state. In contrast, muscarinic receptor blockade with methyl atropine eliminated the bradycardic response of the HR-BRX that was present during 1 MAC isoflurane, and thus, A_2 (lower HR plateau) was increased without affecting A_1 (fig. 3, table 2). The

Intact/sham

A. Atenolol

C. Methyl atropine

Decerebrate
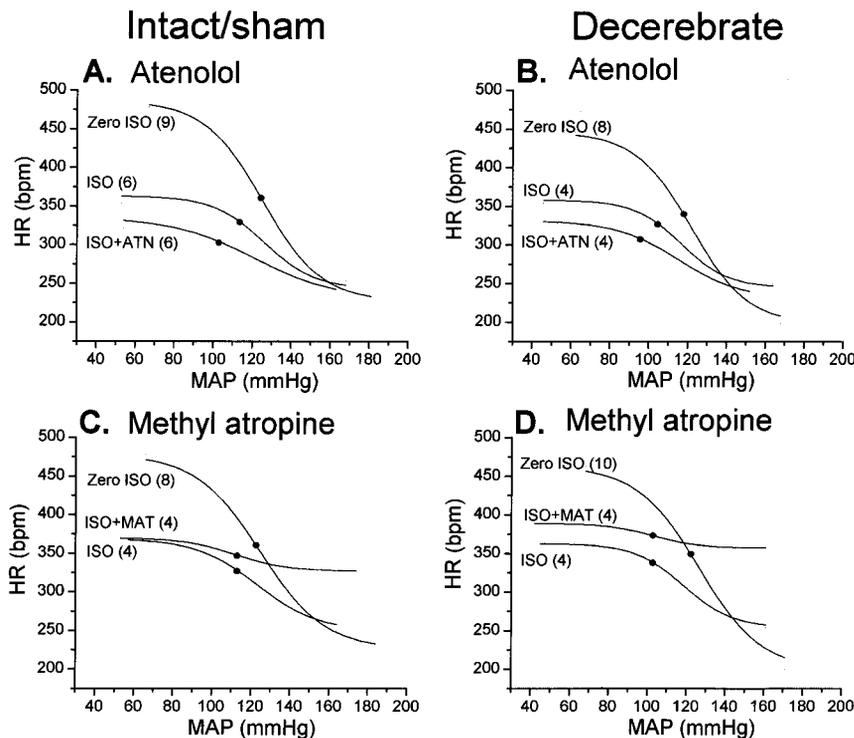
B. Atenolol

D. Methyl atropine

Fig. 3. Autonomic components of 1 minimum alveolar concentration (MAC) isoflurane (ISO) actions on mean arterial pressure (MAP)–heart rate (HR) baroreflex curves for brain-intact/sham (*A* and *C*) and decerebrate (Dec; *B* and *D*) rats. Antagonism of cardiac β-adrenergic receptors with atenolol (ATN; 1 mg/kg) depressed the tachycardia portion of the baroreceptor reflex during isoflurane similarly to isoflurane alone in intact (*A*) and decerebrate (*B*) rats. Antagonism of cardiac muscarinic receptors with methyl atropine (MAT; 0.5 mg/kg) eliminated the bradycardic baroreceptor reflex responses present during isoflurane in intact (*C*) and decerebrate (*D*) rats. Each panel shows curves for the following conditions: (1) zero isoflurane; (2) during 1 MAC isoflurane; and (3) 1 MAC isoflurane plus antagonist. Parentheses indicate the number of animals. Zero isoflurane curves represent pooled averages from other series. Closed circles indicate resting levels of MAP and HR for each curve. bpm = beats/min.

changes in the HR-BRX induced by autonomic blockade in decerebrate rats were similar to those in intact rats.

Discussion

The current studies show that isoflurane depresses the baroreflex control of HR similarly whether suprapontine portions of the brain are present or not. In fact, midcollicular decerebration failed to alter the basic performance characteristics of the HR-BRX compared with the normal, intact state of the conscious animal. Our findings are consistent with the suggestion that fully functional BRX control of HR can be accomplished with CNS structures in or below the pons—likely predominantly within the brain stem and spinal cord.[9,12] To our knowledge, our study is the first to assess the effects of isoflurane on HR-BRX function in decerebrate animals in the absence of basal anesthetics. To place our results in the context of earlier studies, the following sections will consider the technical aspects of assessing the HR-BRX as well as the influence of suprapontine brain structures on autonomic control.

Assessment of the Heart Rate Baroreceptor Reflex

Heart rate baroreceptor reflex depression by isoflurane has been well-documented in awake humans[10] and animals.[7] A technical strength of our studies was the use of vasoactive challenges to evaluate the HR responses over an extensive range of pressures. This method of BRX measurement offers more comprehensive assessment, including direct measurements of the full pressure-response range of the BRX in each condition.[17] The sensitivity of the HR-BRX and the range between the upper and lower plateaus were simultaneously determined in each condition, and time controls proved to be remarkably stable in our within-animal, repeated-measures design. Using logistic fit methods, Bell[11] found similar isoflurane depression of the HR-BRX in fully intact rabbits. Alternative methods used in the majority of HR-BRX studies used a linear regression model to assess BRX gain from HR changes in response to fast ramp increases in systolic arterial pressure.[16,21] Depending on the range and rates of change in the pressure ramps and their relation to the HR plateaus, slope sensitivity and its autonomic subcomponents can differ depending on the rates of injection and pressure changes.[22] Despite these differences in approach, most results in previous isoflurane studies are in general accordance with the current overall findings.[7,10]

Sites of Anesthetic Action

Although our overall results show that suprapontine CNS regulatory sites are not required for anesthetic depression of the HR BRX, they do not identify the anatomic sites or cellular targets by which isoflurane de-

presses the BRX within the full reflex pathways. Isoflurane likely affects multiple components of the BRX pathways. Evidence suggests that these sites may include the afferents, CNS targets, the efferent neurons, ganglionic transmission, and the end organs (heart and blood vessel).[7] Clearly, direct isoflurane depression of cardiac sites, the final common effectors in the BRX, such as the sinoatrial node (e.g., guinea pig),[2] has the potential to dominate HR responsiveness to reflex autonomic inputs. Consistent with such findings, HR decreased with isoflurane in conscious dogs with complete autonomic blockade with propranolol, atropine, and hexamethonium, and such actions were attributed to direct actions on intrinsic cardiac mechanisms.[3] Actions of isoflurane on peripheral vessel smooth muscle should contribute to changes in the control of vascular resistance and systemic hemodynamics.[1] Overall, it is possible that the central and the peripheral actions of isoflurane could have contributed to our observed blunting of the HR-BRX, and this reflects an integrated response to contributions of multiple mechanisms.

Factors Influencing Heart Rate Baroreceptor Reflex Performance

Because isoflurane alters MAP, it is possible that decreases in the prevailing baseline BP during isoflurane could have affected the HR-BRX sensitivity. Some experimental results support this notion: for example, holding BP constant with vasoactive drugs during isoflurane prevented BRX depression (dogs)[7] but had no effect on the isoflurane responses of humans.[10] Considerable work in animals suggests that changes in BP lasting from 15 to 20 min do not change baroreceptor discharge sensitivity, although their pressure activation threshold is reset.[23] At the level of the BRX in conscious subjects without the complication of anesthetic actions, such acute resetting likewise shifts the control of systemic BP[24] and HR[25] to a new set point without affecting BRX slope sensitivity. In rats, we have found that acute changes in BP do not alter BRX sensitivity.[16] Therefore, decreases in BP during isoflurane are unlikely to affect cardiac BRX sensitivity (i.e., gain). Therefore, it is unlikely that pressure per se makes an important contribution to the depression of BRX sensitivity by isoflurane.

Other potential confounding factors that might alter the BRX include increased P_{CO_2},[26] hypoxia,[27] or changes in Na^+ or K^+ plasma concentrations,[28,29] but these remained within normal ranges during isoflurane. Lung inflation by positive pressure can reflexly alter BP and HR depending on the levels of lung inflation,[30] and isoflurane depresses these lung inflation reflexes.[31] However, as with the results of others,[7] substantial differences in breathing pattern (e.g., positive-pressure ventilation during isoflurane compared with spontaneous breathing in the original intact condition) failed to affect isoflurane depression of HR-BRX in our experiments.

Table 2. Baroreflex Parameters in the Presence and Absence of Atenolol or Atropine during 1 MAC Isoflurane Exposure

| | Atenolol (1 mg/kg) | | | | | |
| | Intact–Sham | | | Decerebrate | | |
Group	Zero ISO (9)	ISO (6)	ISO + ATN (6)	Zero ISO (9)	ISO (4)	ISO + ATN (4)
Basal MAP (mmHg)	123 ± 4	113 ± 5*	103 ± 15*	119 ± 13	104 ± 9*	94 ± 6*
Basal HR (beats/min)	357 ± 29	330 ± 34	305 ± 17*	346 ± 30	329 ± 21	310 ± 14
A1 (beats/min)	486 ± 21	363 ± 38*	335 ± 35*	446 ± 38	354 ± 22*	330 ± 14*
A2 (beats/min)	227 ± 26	243 ± 36	230 ± 27	198 ± 48	249 ± 35	239 ± 11
HR range (beats/min)	259 ± 28	120 ± 29*	104 ± 47*	248 ± 29	104 ± 26*	90 ± 25*
BP50 (mmHg)	124 ± 8	126 ± 12	119 ± 16	122 ± 12	117 ± 10	103 ± 6
Slope (beats · min^{-1} · mmHg^{-1})	−3.9 ± 0.9	−2.1 ± 0.6*	−1.3 ± 0.3*	−4.1 ± 1.1	−1.9 ± 0.4*	−1.1 ± 0.6*

Values are mean ± SD. Parentheses indicate the number of animals. Zero isoflurane data are pooled from other animals. Data in the presence and absence of an antagonist during 1 minimum alveolar concentration (MAC) isoflurane exposure are from the same animal.

* Significant difference from Zero ISO ($P < 0.05$).   † Significant difference between ISO + Antagonist ($P < 0.05$).

MAP = mean arterial pressure; HR = heart rate; A1 = upper HR plateau; A2 = lower HR plateau; HR range = difference between upper and lower HR plateau; BP50 = MAP at half HR range; ISO = 1 MAC isoflurane; ATN = atenolol; MAT = methyl atropine.

Central Mechanisms in the Heart Rate Baroreceptor Reflex

The focus of our study was to determine whether supracollicular centers might contribute to alterations in the HR-BRX by isoflurane. Clearly, volatile anesthetics depress medullary cardiovascular centers.[32,33] Direct microinjection of halothane into the medulla in midcollicular decerebrate dogs depressed BP responses to electrical stimulation of medullary pressor and depressor areas.[32] The responses of dissociated NTS neurons to exogenous glutamate were depressed by halothane, whereas responses to γ-aminobutyric acid were enhanced.[33] Isoflurane inhibited BP and HR responses to NTS activation by microinjection of glutamate into medial NTS in decerebrate rats.[34] Together, such results support medullary contributions (*e.g.*, NTS) to cardiac BRX depression during isoflurane.

Although our results show that a highly functional HR-BRX is clearly present in the absence of supracollicular brain regions, considerable evidence supports the potential for contributions by supramedullary cardiovascular centers in autonomic control. Forebrain structures, such as the paraventricular hypothalamic nucleus, lateral hypothalamic area, central nucleus of the amygdala and, bed nucleus of the stria terminalis, are reciprocally connected to the NTS and to the parabrachial nucleus in the pons.[9] These supramedullary centers can importantly impact autonomic function.[9,35] Interestingly, isoflurane[36] and halothane[13] both depress pressor responses evoked by direct activation of the hypothalamus in awake cats. Furthermore, halothane attenuated such pressor responses differentially so that hypothalamic and mesencephalic regions were more sensitive than the medulla.[13] In addition, midcollicular transection increased the susceptibility of the medulla to halothane[13] consistent with a possible inhibitory influence of suprapontine structures on medullary pressor sites. Despite such potential cardiovascular neural actions, BRX pathways in the medulla are depressors, *i.e.*, their activation results in decreases in BP and HR, and therefore, the relation of pressor pathways to BRX depressor mechanisms is less certain. Our results clearly indicate that HR-BRX function is independent of forebrain structures under our conditions. Consistent with this view, Izumi *et al.*[37] demonstrated that isoflurane inhibits parasympathetically mediated reflex vasodilation in the orofacial area (cats) and that decerebration has no effect on isoflurane responses. In the work perhaps most analogous to our study, midcollicular decerebration in cats did not alter isoflurane induced changes in MAP, HR, or autonomic nerve activity compared with the brain-intact condition despite the presence of a basal anesthetic (50% N_2O).[6] Suprapontine modulation on the HR-BRX by various anesthetics may be different. For example, although decerebration did not alter the depression of the HR-BRX by ketamine, HR-BRX depression by althesin was enhanced after decerebration.[12] Because our midcollicular transection eliminated CNS structures such as the hypothalamus, thalamus, and cerebral cortex and their connections to brain structures below the cut, the lack of change in HR-BRX function in our experiments suggests that such modulatory mechanisms were not active either in our completely intact state nor were they engaged by isoflurane treatment. Such results suggest that the mechanisms of isoflurane BRX action reside primarily below the colliculus.

Our midcollicular decerebration did not alter resting MAP, HR, or HR-BRX, a result consistent with many previous reports.[27,38] However, there are several studies reporting a variety of resting MAP and HR values after decerebration even in rats.[18,39,40] No clear pattern of differences in species, age, or preparation seems to be responsible for the differing outcomes. An additional possible factor could include variations in the contribution of the characteristic skeletal muscle rigidity during recovering from anesthesia in decerebrate rats (*i.e.*, ex-

Table 2. Continued

	Methyl Atropine (0.5 mg/kg)				
Intact–Sham			Decerebrate		
Zero ISO (8)	ISO (4)	ISO + MAT (4)	Zero ISO (10)	ISO (4)	ISO + MAT (4)
124 ± 5	110 ± 6*	109 ± 3*	122 ± 9	103 ± 8*	103 ± 7*
355 ± 21	332 ± 25	349 ± 16	353 ± 40	339 ± 37	373 ± 36
478 ± 15	369 ± 27*	370 ± 23*	463 ± 31	363 ± 36*	389 ± 32*
226 ± 27	249 ± 31	327 ± 10*†	200 ± 59	254 ± 41	357 ± 39*†
252 ± 26	120 ± 29*	42 ± 21*†	263 ± 51	109 ± 22*	32 ± 8*†
124 ± 8	126 ± 13	111 ± 4	127 ± 15	119 ± 9	105 ± 13
−3.4 ± 0.5	−1.8 ± 0.7*	−0.8 ± 0.4*†	−3.9 ± 0.7	−1.9 ± 0.4*	−0.6 ± 0.2*†

tension of all limbs, fine muscle fasciculation, and involuntary movement) because such contractions might variably affect resting MAP and HR values.

Autonomic Mechanisms

Our reflex data further suggest that isoflurane depresses primarily sympathetic components of the HR-BRX. This conclusion is based on two primary lines of evidence. First, low concentrations of isoflurane (1 MAC) decreased BRX tachycardia elicited by decreases in BP, whereas reflex bradycardia to increases in BP remained largely unaffected. These bradycardic BRX responses are primarily due to the activation of parasympathetic activity, whereas tachycardic BRX responses often involve both sympathetic activation and vagal inhibition.[17] Such findings are consistent with those of Bell,[11] who reported that isoflurane in awake rabbits depressed the tachycardic responses of the upper plateau more than the bradycardia of the mostly parasympathetically mediated lower plateau of logistic HR-BRX curves. Second, treatment with atenolol, a β-adrenoreceptor antagonist, during 1 MAC isoflurane failed to decrease HR further in response to decreases in BP, indicating nearly complete suppression of sympathetic HR control at this concentration (1 MAC). In contrast, cardiac parasympathetic BRX control was preserved during 1 MAC isoflurane. Atropine reduced the bradycardic responses, showing a substantial parasympathetic component even in the presence of 1 MAC isoflurane. However, 2 MAC isoflurane attenuated the lower HR plateau of the BRX curve consistent with a suppression of BRX parasympathetic activity. Clearly, this high concentration of isoflurane may also recruit a significant contribution of direct cardiac depression.[2] The contribution of such direct effects has not been assessed in the current experiments, and is difficult to evaluate in our system. However, our overall results are generally consistent with reports of sympathetic dysfunction in the CNS vasomotor region and ganglionic transmission[7] and indirect indices of sympathetic function, such as reported decreases in plasma norepinephrine by isoflurane.[41]

Isoflurane produces greater depression of HR responses to sympathetic than to parasympathetic efferent stimulation.[7] However, conversely parasympathetic depression by isoflurane predominates in cats.[6] Isoflurane similarly depresses HR-BRX responses to increases and decreases in pressure in intact dogs, a result consistent with sympathetic and parasympathetic contributions.[7]

Many factors could have contributed to these varied outcomes. Besides technical differences in the experimental protocol (e.g., basal anesthetics and linear and sigmoidal BRX assessments), species differences may play a role in the experimental outcomes. Although isoflurane consistently decreases the prevailing arterial BP, HR is variably altered in different species.[3,10,11,42] Isoflurane decreased HR in awake rats[42] as well as in chloralose-anesthetized rats.[43] In our studies, distinct changes in resting HR were not evident. This may reflect our limited sampling intervals because our purpose was to assess baseline values just before each vasoactive drug challenge (see Methods). Isoflurane is reported to increase HR in awake dogs[3,44] and in humans.[10] Because the HR-BRX responses remain relatively functional during isoflurane (e.g., 1 MAC),[7,10] the resting tachycardia induced by isoflurane in dogs and humans may include a contribution of BRX response to the hypotension in these species. Similar isoflurane concentrations markedly depressed the HR-BRX sensitivity in rabbits[11] and rats in the current study.

In summary, we have demonstrated that isoflurane depressed the HR-BRX function in a similar dose-dependent manner in sham-treated and decerebrate rats. Isoflurane depressed BRX curve parameters derived from logistic curve fits, including sensitivity (slope) and HR range, with a primary effect on the upper HR plateau. The results suggest that isoflurane depresses HR-BRX function by acting on sites at or below pontine CNS regulatory sites. The results do not identify the specific sites of actions within the BRX pathways but likely involve brain stem sites of cardiovascular autonomic regulation. Isoflurane depresses BRX by predominantly inhibiting the sympathetic component of the HR-BRX.

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