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Dexmedetomidine and Regulation of Splenic Sympathetic Nerve Discharge in Aged F344 Rats

RM McMurphy, RJ Fels, and MJ Kenney

Departments of Anatomy and Physiology and Clinical Sciences, Kansas State University, Manhattan, KS.

Abstract

Sedatives influence the immune system and centrally-acting α_2 -adrenergic receptor agonists, including Dexmedetomidine (Dex), modulate sympathetic nerve discharge (SND). Because sedatives are used under medical conditions that include elderly patients, and because advancing age attenuates SND responsivity to various interventions, we tested the hypothesis that splenic sympathoinhibitory responses to Dex would be attenuated in aged compared with young Fischer 344 rats. Dex-mediated reductions in splenic SND were similar in aged and young baroreceptor-intact and -denervated rats, indicating that SND changes to Dex administration occur in an age-independent manner. These findings provide new information regarding interactions between α_2 -adrenergic agonists, advanced age, and SND regulation.

Keywords

Aging; Dexmedetomidine; splenic SND; renal SND; F344 rats

INTRODUCTION

The sympathetic nervous system (SNS) plays a crucial role in regulating physiological homeostasis and central neural circuits regulate sympathetic nerve discharge (SND). Sympathetic nerves innervating many target organs are characterized by a tonic level of background activity and the acute responsivity of the SNS is a critical regulatory feature of this component of the autonomic nervous system. Direct SND recordings provide an output measure of central sympathetic neural circuits and centrally-acting α_2 -adrenergic receptor agonists modulate SND. For example, intravenous Dexmedetomidine (Dex) administration abolishes cocaine-induced increases in skin SND in conscious humans (Menon et al., 2007; Kontak et al., 2013), reduces renal SND in anesthetized rabbits (Oku et

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Correspondence to: Michael J. Kenney, PhD, Department of Anatomy and Physiology, Kansas State University, Coles Hall 228, Manhattan, KS 66506, Phone: 785-532-4513, Kenny@vet.k-state.edu.

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DISCLOSURES

No conflicts of interest are declared by the authors.

al., 1996; Xu et al., 1998), and decreases visceral (splenic and renal) SND in young, anesthetized Fischer 344 (F344) rats (Kenney et al., 2014).

Alpha₂-adrenergic receptor agonists (including Dex) are used as sedative agents for surgical and critically-ill patients (Qiao et al., 2009; Kayhan et al., 2013), and sedatives can influence immune system regulation, supporting sedative-induced immunomodulation (Galley et al., 2000; Taniguchi et al., 2004; Maclaren, 2009; Qiao et al., 2009; Sanders et al., 2009; Kayhan et al., 2013). The nervous system and the immune system communicate via numerous pathways, including the SNS (Kenney and Ganta, 2014). The SNS innervation to the spleen provides a communication pathway between central sympathetic neural circuits and splenic immunocompetent cells (Felten et al., 1985), and changes in splenic SND can influence splenic immune function (Ganta et al., 2004). By modulating splenic SND, centrally-acting alpha₂-adrenergic agonists may impact peripheral immune regulation by altering the function of a secondary lymphoid organ.

The world's population is aging (United Nations, 2004) and advanced age is associated with alterations in SNS regulation, including changes in SND responses to physiological stress (Helwig et al., 2006; Kenney and Fels, 2002; Kenney and Fels, 2003; Kenney, 2010; Seals and Esler, 2000). Critical illness and increased frequency of surgical interventions accompany advancing age, and these medical circumstances may require the use of sedatives to establish and maintain patient comfort. Although sympathoimmune interactions are affected by age-associated alterations (Kenney and Ganta, 2014), the effect of advanced age on the role of central alpha₂-adrenergic receptors in splenic SND regulation is not known.

In the present study the effect of systemic Dex administration on splenic SND and renal SND (visceral nerve control) was determined in aged (22–24 months) and young (3–5 months) male F344 rats. Based on the results of previous studies reporting diminished SND responsivity in aged rats (Helwig et al., 2006; Kenney, 2010; Kenney and Fels, 2002; Kenney and Fels, 2003), we tested the hypothesis that splenic and renal sympathoinhibitory responses to intravenous Dex administration would be attenuated in aged compared with young F344 rats.

METHODS

The procedures and protocols were performed in accordance with the American Physiological Society's guiding principles for research involving animals and approved by the Institutional Animal Care and Use Committee at Kansas State University.

General Procedures

Experiments were completed in young (3–6 months old; 348±21 g) and aged (22–24 months old; 424±10 g) male F344 rats (Charles River Laboratories, contracted with the National Institute on Aging). Anesthesia was induced by isoflurane (3–5%; Butler Animal Science) and maintained during surgical procedures using isoflurane (1.5%–2.5%), α-chloralose (80 mg/kg, ip; Sigma), and urethane (800 mg/kg, ip; Sigma) (Kenney et al., 2014). Maintenance doses of α-chloralose (35–45 mg/kg/hr) were administered intravenously and maintenance

doses of urethane (200 mg/kg every 4 hours) were administered into the intraperitoneal space. The trachea was cannulated and rats were paralyzed with gallamine triethiodide (5–15 mg/kg iv, initial dose; 10–15 mg/kg/hr, maintenance dose; Sigma) and artificially ventilated (Kenney et al., 2011). End-tidal CO₂ was measured using a micro-capnometer (Columbus Instruments) and was maintained near 4.5% during experimental procedures. Isoflurane anesthesia was discontinued following surgical procedures. Femoral arterial pressure was recorded (Digi-Med BPA) and heart rate (HR) was derived from the pulsatile arterial pressure output of the blood pressure analyzer. Colonic temperature (T_c) was kept at 38.0°C by a temperature-controlled table.

To eliminate the influence of baroreceptor afferent feedback mechanisms that can alter SND responses of central origin, a subset of experiments were completed in sino-aortic denervated (SAD) rats. Denervations were completed using standard procedures 3–4 hours before initiation of experimental protocols (Hirai et al., 1995). Denervation was considered complete by the absence of reflex SND changes during pharmacological-induced changes in mean arterial pressure (MAP).

Sympathetic Nerve Recordings

Activity was recorded biphasically with a platinum bipolar electrode after capacity-coupled preamplification (bandpass 30–3000 Hz) (Grass Instruments) from renal and splenic sympathetic nerves (Kenney et al., 2014). Filtered neurograms were full-wave rectified and integrated (time constant 10 ms) and SND was quantified as microvolts × seconds (μV·s) (Kenney et al., 2014). SND recordings were corrected for background noise after ganglionic blockade (chlorisondamine, 5 mg/kg iv; Sigma) (Kenney et al., 2014). The adequacy of anesthesia was demonstrated by an inability of mechanical stimulation of the hindlimb or tail to increase MAP or SND.

Experimental Protocols

Pre-injection control levels of MAP, HR, splenic SND, and renal SND were recorded for 15 min and control values were determined from the average of the final 5 min (baseline is represented as time 0 in Figures 1 and 2). Rats received Dex (1.0 μg/kg, iv) dissolved in 300 μl saline or vehicle (saline 300 μl, iv). The dose of Dex used in the present study was selected based on a range of Dex doses used in a recent study involving human subjects (Kontak et al., 2013). In the first experimental series aged and young baroreceptor-intact (Intact) rats received either Dex (aged, n=10; young, n=6) or saline (aged, n=7; young, n=3). In the second experimental series aged SAD rats received Dex (n=7) and responses were compared to the aged Intact rats (n=10) from the first experimental series. At the end of experiments rats were euthanized via an overdose of methohexital sodium (Brevital, 150 mg/kg, iv; JHP Pharmaceutical).

Data and Statistical Analysis

A computer-based ADInstruments Powerlab data acquisition system was used to collect experimental data. Values are means ± SE. MAP (mmHg) and HR (beats per minute, bpm) data are reported as absolute change from baseline values (control). The level of background signal remaining after ganglionic blockade of efferent SND with chlorisondamine was

subtracted from the full signal. Baseline levels of splenic SND and renal SND were considered as 0% after subtraction of the background signal. SND data are reported as percent change from control. Statistical analyses included Student *t*-tests for pairwise comparisons and ANOVA techniques with a repeated-measures design followed by Bonferroni post hoc corrections. The level of statistical significance was $p < 0.05$.

RESULTS

Traces of pulsatile arterial blood pressure, splenic SND (original and integrated traces), and renal SND (original and integrated traces) from representative young (A) and aged (B) Intact F344 rats during control, 10 and 45 min after intravenous Dex administration (1 $\mu\text{g/kg}$), and following ganglionic blockade are shown in Figure 1. Arterial blood pressure, splenic SND, and renal SND were decreased from control levels 10 min after Dex, demonstrated variable levels of recovery at 45 min of recovery, and were reduced in response to ganglionic blockade in the young F344 rat (Figure 1A) and in the aged F344 rat (Figure 1B).

Control levels of MAP (aged, 81 ± 3 mmHg; young, 114 ± 5 mmHg) and HR (aged, 354 ± 4 bpm; young, 424 ± 6 bpm) were significantly lower in aged compared with young Intact F344 rats. MAP, HR, splenic SND, and renal SND responses from control levels (time 0) recorded for 45 min after intravenous administration of Dex (1 $\mu\text{g/kg}$: aged, $n=10$; young, $n=6$) or saline (aged, $n=7$; young, $n=3$) in Intact F344 rats are summarized in Figure 2A. Dex produced transient (2 min) and significant increases in MAP in aged and young rats (designated collectively by the single cross), followed by significant reductions in MAP from control levels in both groups (designated collectively by the double asterisk) (Figure 2A). In both aged and young rats MAP remained at nadir levels for approximately 10–15 min after Dex administration, followed by progressive recovery towards control levels (Figure 2A). HR was significantly reduced from control levels in response to Dex in both aged and young rats (designated collectively by the double asterisk), and with the exception of the 5-min recovery point, the Dex-mediated bradycardia did not differ between aged and young rats (Figure 2A). Splenic SND and renal SND were significantly reduced from control levels following Dex administration in aged and young rats (designated by the double asterisks), and the magnitude of the sympathoinhibitory responses did not differ between groups (Figure 2A). In both young and aged rats renal and splenic SND remained at nadir levels for approximately 10–15 min, followed by progressive recovery towards control values (Figure 2A). MAP, HR, splenic SND, and renal SND remained unchanged from control values for 45 min after intravenous saline administration in aged and young rats (Figure 2A).

Cardiovascular and SND responses from control levels (time 0) recorded for 45 min after intravenous Dex administration (1 $\mu\text{g/kg}$) in Intact (same data as Figure 2A) and SAD ($n=7$) aged F344 rats are summarized in Figure 2B. Baseline MAP was 89 ± 3 mmHg and baseline HR was 361 ± 5 bpm in SAD aged F344 rats. Dex administration produced similar responses in aged SAD and Intact rats; transient increased (designated collectively by the single cross) followed by reduced MAP, bradycardia, and splenic and renal sympathoinhibition (significance designated by the double asterisks for each variable) (Figure 2B). Splenic SND and renal SND were significantly reduced from control following Dex in aged SAD and

Intact rats, and the nadir of the sympathoinhibitory responses did not differ between groups (aged SAD vs. aged Intact) (Figure 2B).

DISCUSSION

In a recent study we reported that intravenous Dex administration produced splenic and renal sympathoinhibition in young F344 rats (Kenney et al., 2014). Because sedatives are often used under medical conditions that include elderly patients, coupled with the consideration that advancing age alters SND responsiveness (see Kenney 2010), it was plausible to test the hypothesis that visceral sympathoinhibitory responses to intravenous Dex administration would be attenuated in aged compared with young F344 rats. However, in contrast to the stated hypothesis, the present results indicate Dex-induced reductions in splenic and renal SND are similar in baroreceptor-intact aged and young rats. Consistent with the response profile in young F344 rats (current results and Kenney et al., 2014), Dex administration in the aged rats produced a short-lasting increase in MAP, an effect that may have contributed to the visceral sympathoinhibitory responses secondary to activation of the arterial baroreflex. However, Dex-mediated reductions in splenic and renal SND were observed in SAD aged F344 rats, and the sustained Dex-induced hypotensive responses in the intact aged F344 rats were not accompanied by visceral sympathoexcitatory responses, suggesting that neither activation nor unloading of the arterial baroreceptors contributed substantively to the Dex-induced SND responses. Collectively, these data support the finding that Dex administration produces centrally-mediated changes in visceral sympathetic nerve outflow in aged F344 rats.

It is widely accepted that advancing age alters several important aspects of SNS regulation in human subjects, including; sympathetically-mediated support of arterial blood pressure regulation, alterations in the background level of muscle and skin sympathetic nerve activity, and SNS responsivity to various acute stressors (Seals and Esler, 2000). In addition, SND regulation is modified with advancing age in animal models. Acute heating provides a potent stimulus for activation of visceral SND in young but not aged F344 rats (Kenney and Fels, 2002; Kenney and Fels, 2003), whereas sympathoinhibitory responses to hypothermia are attenuated in aged compared with young F344 rats (Helwig et al., 2006). The present results demonstrate that Dex administration produced reductions in sympathetic nerve outflow directed to the spleen and kidney in both aged and young F344 rats, supporting the idea that activation of central α_2 -adrenergic receptors and subsequent alterations in efferent visceral SND occur in an age-independent manner. In addition, and similar to findings in young rats, the fact that Dex administration produced sympathoinhibitory responses in both splenic and renal sympathetic nerve activity in aged rats suggests that α_2 -adrenergic agonists may produce a generalized attenuation in peripheral sympathetic nerve outflow, at least with regards to visceral SND. Importantly, changing the level of efferent SND is a regulatory strategy whereby the SNS modulates target organ function.

The SNS plays an important role regulating physiological homeostasis and responding to acute stressors, and recent evidence indicates the SNS is involved in mediating interactions between the nervous system and the immune system (Kenney and Ganta, 2014). The results of several recent studies support the idea that Dex administration can reduce levels of

immune cell products (pro-inflammatory cytokines, and interleukins) and apoptosis factors (Qiao et al., 2009), and modulate immune regulation in septic and colitis-induced rats (Qiao et al., 2009; Kayhan et al., 2013). It has been hypothesized these drugs may influence immune function by modulating the SNS. The SNS innervation to the spleen serves as an important communication pathway between the central nervous system and splenic immunocompetent cells (Felten et al., 1985; Felten et al., 1987), and alterations in the level of splenic sympathetic nerve activity can modulate splenic cytokine gene expression (Ganta et al., 2004). The present study is the first to determine the effects of Dex on splenic nerve outflow in aged F344 rats, and based on previous studies indicating that changes in sympathetic nerve outflow can influence splenic cytokine gene expression (Ganta et al., 2004; Ganta et al., 2005), it must be considered that Dex-induced inhibition of splenic nerve outflow may affect splenic immune function in aged subjects. Moreover, altering the level of renal SND can markedly modulate physiological status, as the sympathetic neural innervation to the kidney influences physiological parameters involved in blood pressure regulation, including renal blood flow, renin release, and salt and water retention by the renal tubules (DiBona, 1994). Although additional studies are required to determine the extent of Dex-induced changes on splenic cytokine gene expression and cytokine levels, as well as renal function, the present results suggest that peripheral administration of a centrally-acting α_2 -adrenergic agonist can influence visceral SND in aged rats which in turn may affect splenic and kidney function.

Dex is often administered to patients whom have received other analgesic, anesthetic or sedative agents; therefore the completion of the present experiments using an anesthetized preparation is not inconsistent with the clinical use of this agent. The anesthetic protocol employed has been used extensively in experimental preparations involving SND recordings. Both young and aged rats demonstrated similar cardiovascular and SND responses to intravenous Dex administration, suggesting the lack of an anesthetic-associated, age-related influence on Dex responsivity. On the other hand, basal levels of MAP and HR were significantly lower in aged compared with young F344 rats. It cannot be discounted that the anesthetized state or the specific anesthetic protocol employed may contribute to age-related differences in basal cardiovascular regulation. The level of activity recorded in multifiber SND preparations is influenced by several methodological factors, making the interpretation of the absolute level of SND problematic. Therefore, SND responsiveness is typically reported as percent change from control, which raises concern when comparing SND responses between groups of animals such as young and aged rats. This is a limitation of the present study, although it is important to note that in aged rats Dex but not saline administration reduced visceral SND (splenic and renal), demonstrating the SND regulatory significance of α_2 -adrenergic agonists in aged animals irrespective of the comparison to young animals.

Further understanding of regulatory relationships between aging, Dex administration, visceral sympathetic nerve outflow, and ultimately visceral target organ function, may assist in informing the use of Dex or other central acting α_2 -adrenergic agonists as adjunct sedatives in aged patients.

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HIGHLIGHTS

- Sedatives affect immune function and the sympathetic nervous system (SNS).
- Splenic sympathetic nerve discharge links the SNS and splenic immune function.
- Regulation of sympathetic nerve discharge is altered with advanced age.
- Effect of Dexmedetomidine (Dex) on splenic nerve discharge explored in aged rats.
- Dex administration reduced splenic nerve discharge in aged and young F344 rats.

Figure 1.

Traces of pulsatile arterial blood pressure, splenic SND (original and integrated traces), and renal SND (original and integrated traces) from representative young (A) and aged (B) F344 rats with intact arterial baroreceptors during control, 10 and 45 min after intravenous Dex administration (1 μ g/kg), and following ganglionic blockade. Horizontal calibration is 250 ms.

Figure 2.

(A): Responses of mean arterial pressure (MAP, mmHg), heart rate (HR, bpm), splenic sympathetic nerve discharge (SND, %), and renal SND (%) recorded for 45 min after intravenous administration of Dexmedetomidine (Dex) (1 µg/kg) or saline in baroreceptor-intact aged (Dex: closed circles, n=10; Saline, n=7) and young (Dex: open circles, n=6; Saline, n=3) rats. Baseline levels are represented as Time 0. †MAP significantly increased from control levels in response to Dex administration in both aged and young rats. *HR significantly different between aged and young rats at the 5 min point following Dex

administration. **MAP, HR, splenic SND, and renal SND significantly reduced from control levels in response to Dex administration in both aged and young rats. (**B**): Responses of mean arterial pressure (MAP, mmHg), heart rate (HR, bpm), splenic sympathetic nerve discharge (SND, %), and renal SND (%) recorded for 45 min after intravenous administration of Dexmedetomidine (Dex) (1 µg/kg) in baroreceptor-intact (closed circles, n=10) and sinoaortic-denervated (SAD) (open circles, n=7) aged rats. Baseline levels are represented as Time 0. †MAP significantly increased from control levels in response to Dex administration in both Intact and SAD aged rats. **MAP, HR, splenic SND, and renal SND significantly reduced from control levels in response to Dex administration in both Intact and SAD aged rats.