Decentralization of the Superior Cervical Ganglia and the Immediate Hypersensitivity Response (43468)

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Abstract. **Bilateral decentralization of the superior cervical ganglia protects against pulmonary inflammation when measured 8 hr after induction of anaphylaxis in rats sensitized to the nematode, *Nippostrongylus brasiliensis*. Since anaphylactic shock produces immediate perturbations to the cardiovascular and respiratory systems, we examined whether bilateral decentralization of the superior cervical ganglia modified the responses of these two systems during the first 4 hr of the anaphylactic response. With the exception of the bronchioles, decentralization did not protect against anaphylaxis-associated increases in extravasation of albumin, and the small changes in respiratory function induced by anaphylaxis were unaffected by the denervation. Decentralization did not alter anaphylaxis-induced reductions in blood flow to the gastrointestinal tract; however, blood flow to the kidneys and spleen of decentralized rats was restored more rapidly to normal values. These results suggest that the protective effect of decentralization on the late phase pulmonary inflammation of anaphylaxis is unrelated to early changes in respiratory mechanics, although the protection may be facilitated by the more rapid re-establishment of normal cardiovascular homeostasis.**

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A severe systemic reaction known as anaphylactic shock occurs when a sensitized animal is exposed to the specific antigen. This hypersensitivity reaction is caused by immunologic mediators that affect smooth muscle and cause vascular collapse and/or airway constriction (1, 2). In response to this marked perturbation of normal homeostasis, numerous components, including the central nervous (3), hypothalamopituitary (4), and sympathetic nervous systems (5, 6), are rapidly summoned to maintain life-support mechanisms and to re-establish normal function of vascular, pulmonary, and immune systems.

During our investigations of neural regulation of immune system function during anaphylaxis in rats, we observed that the cervical sympathetic trunk modulates the severity of hypersensitivity reactions (7). Specifically, decentralization of the superior cervical ganglia (SCG) reduced the degree of pulmonary inflammation evident 8 hr after induction of anaphylaxis. This response occurs, in part, through the suppression of the phagocytic and chemotactic properties of neutrophils (8), cells which contribute substantially to the deterioration of tissue and organ function in various types of vascular injury (9). In the present study, we examined the effect of decentralization of the SCG on immediate hypersensitivity responses by monitoring respiratory and cardiovascular function after antigen challenge in *Nippostrongylus brasiliensis*-sensitized rats.

Methods

Animals, Infections, and Surgical Procedures. Male and female Sprague-Dawley rats (Charles River Breeding Laboratories, Canada) of an initial weight of 200–250 g were maintained in filter-top cages (two to three rats per cage) to minimize the possibility of unwanted infections. Rats were sensitized to the nematode *N. brasiliensis* by infection with 3000 third-stage larvae (7, 10). The sensitization and surgical procedures were identical to those used in a previous study (7), in which

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we reported that bilateral decentralization of the SCG markedly reduces the pulmonary inflammation that develops 8 hr after induction of anaphylaxis.

Surgical procedures were performed 30 days after infection under halothane anesthesia. A bilateral decentralization of the SCG was performed by removing a 3-mm segment of the nerve trunk joining the middle and superior cervical ganglia. In the rat, the cervical sympathetic trunk runs as a small nerve, distinct from the vagus, on the inferior side of the carotid artery. The success of the decentralization was determined by the loss of motor tone to the eyes and the development of bilateral ptosis and miosis. The sham operation consisted of exposing the cervical sympathetic trunk, but no nerves were cut. A final group of animals which received no surgical manipulation served as unoperated controls. In several experiments, uninfected animals were studied as well.

Seven days after surgery, the animals were anesthetized with either halothane or pentobarbital (55 mg/kg). All animals, sensitized and unsensitized, were injected via the penile vein with 100 μl of antigen containing 150 worm equivalents of homogenized *N. brasiliensis*.

Measurement of Ventilation. Ventilation was measured using a modified plethysmographic technique described previously (11). Awake rats were placed in a 7-liter Plexiglass plethysmograph and respiration was indicated on an polygraph by oscillations in pressure proportional to tidal volume. The thermal changes in the experimental chamber, associated with wetting and thermal expansion of the inspired air and drying and cooling of the expired air, were measured by a differential pressure transducer (Validyne MP-45, ±2 cm H_2O) referred to an identical sealed chamber containing room air. Pressure differences between the animal and reference chambers were followed for 10 to 20 sec by occluding the inlet and outlet valves of the chamber. After each measurement period, a dynamic calibration of the recording chamber was made by injecting 0.3 ml of room air into the experimental chamber at the end of an expiration. Tidal volume was calculated using the modified equation described by Bartlett and Tenney (11).

When stable baseline values were obtained, generally after 1 hr in the chamber, the rat was briefly anesthetized with halothane and the antigen (150 worm equivalents) was administered. The animal was quickly reintroduced into the plethysmograph and ventilatory measurements were taken at 5, 10, 15, 20, 25, 30, 45, 60, 75, 90, 105, and 120 min.

Measurement of Plasma Extravasation. Changes in permeability to serum albumin were analyzed using Evans blue (12) in rats anesthetized with pentobarbital. The right jugular vein was cannulated for injection of Evans blue (20 mg/kg) in saline (20 mg/ml). Plasma extravasation was studied at either 5 min or 60 min after antigen challenge. Five minutes after dye injection, the thorax was opened and the animals were perfused via the left ventricle with 50 ml of saline at 80 cm of water pressure. Various tissues were excised, weighed, and placed in 2 ml of formamide for extraction of the dye for 72 hr at room temperature (20°C). The amount of Evans blue in the formamide was colorimetrically measured with a Beckman DU-50 spectrophotometer at 620 nm.

Blood Flow Measurements with Radiolabeled Microspheres. Blood flows were determined at three time points (5, 60, and 240 min) after intravenous injection of antigen in separate groups of pentobarbital-anesthetized rats. The left ventricle was cannulated through the right carotid artery with PE-50 tubing for the administration of microspheres and the right femoral artery was cannulated (PE-50) for monitoring blood pressure (BP) and heart rate (HR). Ventricular placement of the cannula was verified by the characteristic left ventricular pressure tracing obtained.

Regional blood flow was determined by injecting approximately 80,000–100,000 microspheres labeled with ^{113}Sn (15 ± 3 μm, sp act 10 μCi/g; New England Nuclear). The microspheres, suspended in 0.9% NaCl containing 0.05% Tween 80, were thoroughly mixed before injection into the left ventricle in a total volume of 0.5 ml, with 0.8 ml of saline flush. Starting 15 sec before the microsphere injection, the reference blood sample was drawn into a motor-driven syringe at a rate of 0.68 ml/min for 75 sec.

Several min after injection of microspheres the animals were sacrificed with an overdose of urethane. Individual tissues were removed, weighed, and counted on a dual-channel LKB gamma counter. Data reductions were performed with the program described by Flaim and co-workers (13). Results are expressed in terms of blood flow per gram of wet tissue (ml/min/g).

Blood Velocity Measurements with the Microdoppler Technique. A modification of the technique developed by van Orden and co-workers (14) was used. In pentobarbital-anesthetized rats, a laparotomy was performed and microdoppler cuffs of the appropriate size were placed on the left renal artery (LRA) and superior mesenteric arteries. The right carotid artery was cannulated for monitoring HR and BP. The right jugular vein was cannulated for the continuous infusion of saline (2 ml/hr) and the administration of antigen.

After 1 hr of stabilization, the animals were injected with antigen, and changes in BP, HR, LRA, and superior mesenteric artery blood flow were followed for 1 hr.

Statistical Analysis. All data values are presented as the mean ± SE. The variability in the data between the different groups of animals was compared using two-way multivariate analysis of variance (treatment

and time), with post hoc analysis using the Student-Newman-Keul procedure (15). For the respiration studies, differences between groups were analyzed with Student's t test. Differences were considered significant if $P < 0.05$.

Results

Respiratory Function. Respiratory function in unanesthetized sham-operated and decentralized rats was monitored for 2 hr after the administration of antigen. The onset of anaphylaxis, which occurred within a few minutes of injection of the antigen (see blood flow studies below), was characterized by a small, but not significant, increase in minute ventilation at 10 min after antigen injection (Fig. 1) in both sham-operated and decentralized animals. No statistical differences in minute ventilation between the two groups of animals were observed. Neither frequency of breathing nor breath duration was affected by the anaphylactic response in sham-operated and decentralized animals (results not shown).

Plasma Extravasation Measurements. Five minutes after the administration of antigen, marked albumin leakage occurred in the trachea, bronchioles, tongue, and jejunum, but not in the kidney and liver (Table I). One hour after antigen injection, albumin leakage had returned to levels seen in unsensitized animals for the majority of tissues, except for the bronchioles and trachea, which still exhibited a 4- to 5-fold increase in permeability for both sham-operated rats. Decentralization generally did not prevent the increase in albumin permeability, except in the bronchioles, where it was restored to control levels at 60 min after the induction of anaphylaxis.

Blood Flow Changes Associated with Decentralization. Multifactorial analysis of variance indicated that blood flows to the spleen, kidneys, brain, stomach, and jejunum, but not the heart, were modified in the four groups of animals over time by intravenous injection of antigen (Fig. 2). Anaphylaxis in sensitized rats initially (5 min after antigen) reduced blood flow to the brain and splanchnic organs (e.g., spleen, stomach, and jejunum), whereas flow to the heart and kidneys was maintained (Fig. 2). Four hours after onset of anaphylaxis, blood flow was similar in all groups for the majority of tissues, although a trend of reduced blood flow to the jejunum was apparent. With most tissues, decentralization did not substantially alter blood flow values from those seen in the unoperated or the sham-operated rats at any of the time periods tested (e.g., jejunum). However, at 1 hr after antigen administration, enhanced blood flows were observed to the spleen and kidneys in decentralized rats, when compared with unoperated and sham-operated rats.

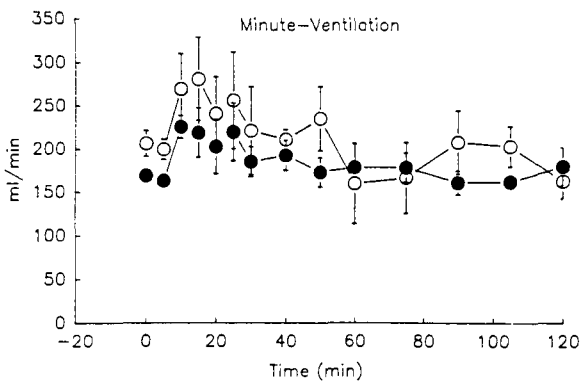

Figure 1. Minute ventilation measured for 120 min after injection of antigen at 0 min in decentralized (○—○; $n = 4$) and sham-operated (●—●; $n = 4$) sensitized rats. Four rats were used in each group.

Upon the induction of anaphylaxis, an immediate drop in blood flow in the superior mesenteric artery occurred that persisted for 60 min (Fig. 3; $P < 0.05$) and was not altered by decentralization or sham operation. Blood flow in the LRA also exhibited a pronounced decrease with antigen administration which persisted for 60 min, although the decrease in blood flow was significantly less in the decentralized rats when compared with the unoperated animals. For sham-operated rats, the blood flow in the LRA was intermediate between that measured in decentralized and unoperated animals.

Mean arterial BP and HR were monitored in these experiments for 60 min after antigen administration. The onset of anaphylaxis was associated with an initial decrease in BP 10 min after antigen injection, which tended to recover and then remain relatively stable over the next 50 min. No significant differences in HR and BP were observed among the unoperated, sham-operated, and decentralized animals after the administration of antigen.

Discussion

Several investigators have suggested that the intestine is the major anaphylactic organ in the *N. brasiliensis*-sensitized rats (16–18), and our blood flow studies confirm this suggestion. Respiratory dysfunction did not occur during the immediate hypersensitivity response in the *N. brasiliensis*-sensitized rats (Fig. 1). Blood flow to the intestine was dramatically reduced for at least 1 hr, whereas blood flows to the trachea and bronchioles (7), as well as the brain (Fig. 2), were back to normal within 1 hr of administration of antigen. Anaphylaxis is associated with marked extravasation of serum albumin in the respiratory and digestive systems, and the associated fluid accumulation in the intestinal lumen indicates massive secretion of water during anaphylaxis.

Decentralization of the SCG (7), which protects against the development of pulmonary inflammation 8 hr after the induction of anaphylaxis, does not protect against early anaphylactic hypotension (Fig. 3). Fur-

Table I. Evans Blue Measurements 5 and 60 Min after Antigenic Challenge in *Nippostrongylus brasiliensis*-Sensitized Rats[a]

Tissue	Unsensitized	5 min		60 min	
		Sham	Decent	Sham	Decent
Kidney	18.8 ± 2.8	38.4 ± 8.1	23.4 ± 8.6	42.1 ± 15.6	24.6 ± 7.6
Liver	12.2 ± 3.0	15.0 ± 3.6	9.4 ± 1.3	11.6 ± 5.9	12.7 ± 4.6
Trachea	5.9 ± 0.7	106.1 ± 20.7[b]	95.7 ± 10.7[b]	25.2 ± 5.2[b]	18.0 ± 3.7[b]
Bronchioles	13.2 ± 2.4	55.2 ± 11.1[b]	67.5 ± 19.5[b]	37.8 ± 9.8[c]	15.9 ± 3.1
Jejunum	11.9 ± 3.8	62.3 ± 7.4[b]	56.1 ± 4.5[b]	14.1 ± 2.3	11.7 ± 4.1
Tongue	2.2 ± 0.4	9.1 ± 2.0	8.6 ± 3.7	3.9 ± 0.8	4.8 ± 1.4

[a] Values are mean ± SE for ng of Evans blue/mg wet tissue. Sham, sensitized and sham-operated; decent, sensitized and decentralized. Number of animals: unsensitized, $n = 6$; sham at 5 min, $n = 6$; decent at 5 min, $n = 6$; sham at 60 min, $n = 5$; decent at 60 min, $n = 5$.
[b] >unsens; $P < 0.05$.
[c] Sham > unsens, decent; $P < 0.05$.

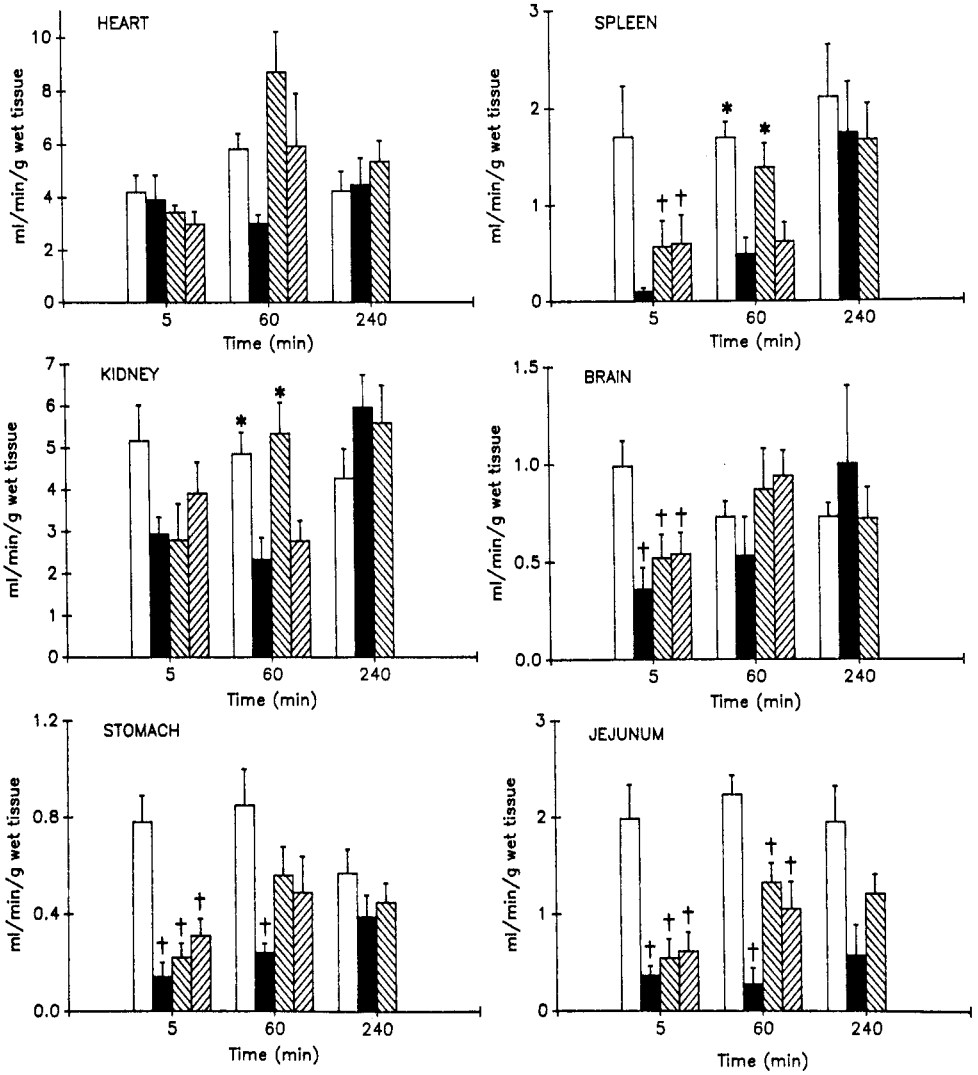

Figure 2. Blood flows in ml/min/g wet tissue for the heart, spleen, kidney, brain, stomach, and jejunum at 5, 60, and 240 min after antigen injection. One group of animals was unsensitized (□; $n = 9$), whereas the other three groups (unoperated [■; $n = 7$], decentralized [▨; $n = 5$], and sham operated [▧; $n = 7$]) were sensitized and treated. Levels of significance ($P < 0.05$): †group less than unsensitized group; *greater than unoperated and sham-operated groups.

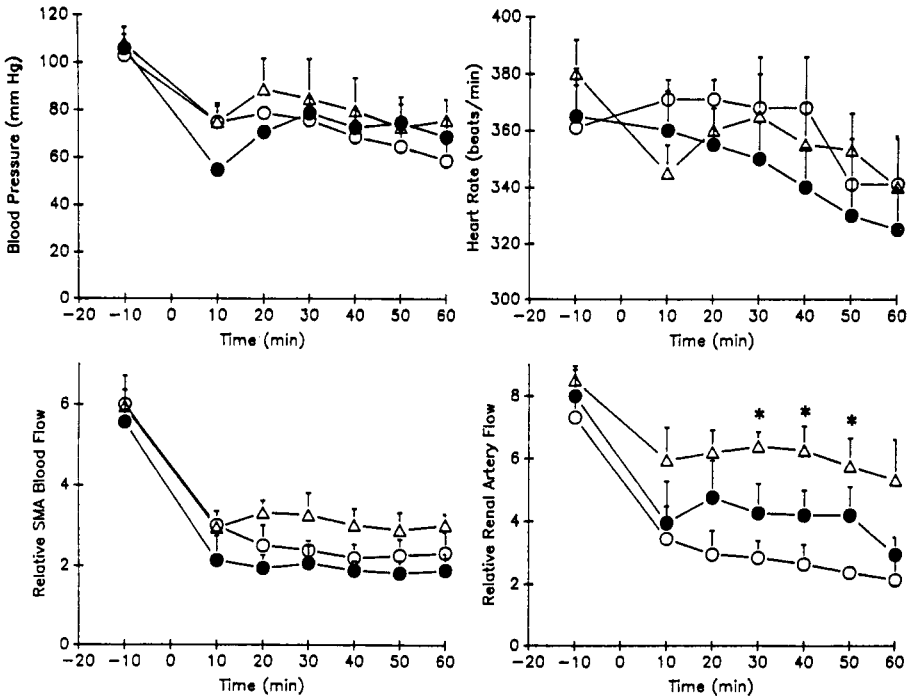

Figure 3. Blood pressure, heart rate, and relative flows in superior mesenteric artery (SMA) and renal artery in unoperated (○—○; $n = 4$), decentralized (△—△; $n = 4$), and sham-operated (●—●; $n = 4$) sensitized rats in response to antigen administration at $t = 0$ min. Levels of significance ($P < 0.05$): *greater than unoperated rats.

thermore, blood flow changes to the brain, gastrointestinal tract (Fig. 2), or trachea and bronchioles (7) are modified to a similar extent in unoperated, sham-operated, and decentralized animals after antigen challenge.

It is well recognized that sympathetic outflow, necessary for the maintenance of vascular tone, is seriously compromised during anaphylaxis (5). Koyama and coworkers (19) have recently demonstrated that systemic baroreceptor reflex control of renal nerve activity is reduced during anaphylactic hypotension, and this decrease is independent of baroreceptor activation. These authors proposed that chemical mediators of anaphylaxis affect neurons in the central nervous system that regulate sympathetic outflow. Decentralization may prevent the anaphylaxis-induced reduction in sympathetic outflow from the central nervous system, but this peripheral sympathetic response is only apparent in selected tissues (e.g., spleen and kidneys) in these rats.

An alternative, and possibly complementary, explanation may reside in the differential responses of the immune system of decentralized animals and sham-operated animals, especially for the neutrophil (see below). The hallmark of immediate hypersensitivity reactions is the activation of mast cells, with the release of preformed and newly synthesized mediators such as histamine (20) and leukotrienes (21). The pronounced initial perturbations in cardiovascular function that occur equally in unoperated, decentralized, and sham-operated animals, suggest that early systemic hypersensitivity reactions are not altered by decentralization.

Neutrophils probably play a significant role in determining the severity of late hypersensitivity reactions (22, 23). We observed a marked pulmonary neutrophilia 8 hr after injection of antigen, and found that this response is markedly depressed in decentralized animals (7). This depression of pulmonary neutrophilia is probably due to attenuated chemotactic and phagocytotic responses by neutrophils in decentralized animals (8), and may contribute to reduced plasma extravasation of the bronchioles in decentralized rats 60 min after antigen challenge (Table I). Activated neutrophils release a large number of enzymes which cause extensive tissue damage and play a critical role in ischemia-induced vascular injury (24). Recent studies by Altura and Gebrewold (25) have demonstrated that the cells of the reticuloendothelial system serve to modulate microvascular tone and vascular reactivity. These investigators noted that upon blockade of the reticuloendothelial system, mesenteric terminal arterioles exhibit a hyperreactivity to the vasoconstrictor noradrenaline and hyporeactivity to the dilator acetylcholine. This type of mechanism may be operating in decentralized animals, and may play an important role in modulating vascular reactivity, especially in the spleen and kidneys, during the immediate hypersensitivity reaction.

Although the mechanism by which decentralization of the SCG protects against anaphylaxis remains

unknown, an endocrine mechanism has been proposed. We have found that removal of the submandibular glands in male rats abolishes the protective effect of decentralization against anaphylaxis (26), and we hypothesize that decentralization of the SCG regulates the circulating levels of the anti-inflammatory factor(s) released from this gland. The SCG innervates the submandibular glands, an important source of several immunoregulatory activities (27). We have recently found (unpublished observations) that removal of the submandibular glands (sialoadenectomy) prevents the down-regulation of neutrophil function observed with decentralization.

Thus, we have shown that decentralization of the SCG, which attenuates the pulmonary inflammation that develops 8 hr after antigen challenge in *N. brasiliensis*-sensitized rats (7), did not prevent all of the hemodynamic changes that occur with the onset of anaphylaxis. Nevertheless, the reduced extravasation in the bronchioles and the restoration of normal blood flow to the kidneys and spleen in the decentralized rats may contribute to a more rapid re-establishement of normal pulmonary and cardiovascular homeostasis in the shocked animals.

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