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Tonic Sympathoinhibition in the Baroreceptor Denervated Cat[1] (40114)

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The baroreceptor reflexes have long been recognized as having a critical role in controlling the level of sympathetic nerve discharge (SND) and, thereby, maintaining blood pressure within normotensive levels (1). Sympathoinhibitory mechanisms of nonbaroreceptor origin apparently also function tonically to control SND. Alexander (2) reported an increase in SND after cervical spinal cord section in an animal in which SND had previously been maximally reduced by medullary transection at the level of the obex. The increase in SND presumably was due to release of inhibition of nonbaroreceptor origin since transection at the level of the obex is caudal to the point of entry into the medulla of the IX and X cranial nerves. Coote *et al.* (3) showed that the spinal component of the somatosympathetic reflex was augmented after section of the cervical spinal cord. It was suggested that the enhancement of the reflex was due to the release of inhibition of nonbaroreceptor origin since these investigators also reported that the reflex was not affected by baroreceptor reflex activation in cats with an intact neuraxis.

The present investigation was designed to answer three questions regarding sympathoinhibition of nonbaroreceptor origin. First, what are the sources of tonic sympathoinhibition in the baroreceptor denervated cat? Second, is tonic sympathoinhibition of nonbaroreceptor origin transmitted over a brainstem circuit distinct from that which receives baroreceptor input? Third, what are the relative contributions made by the nonbaroreceptor and baroreceptor inhibitory systems in the control of SND in the anesthetized cat?

Materials and methods. Cats weighing between 2.0 and 3.5 kg were anesthetized with an ip injection of a mixture of sodium diallylbarbiturate (60 mg/kg), urethane (240 mg/kg), and monoethylurea (240 mg/kg). Animals were paralyzed with gallamine triethiodide (4 mg/kg, iv) and artificially respired in order to prevent somatomotor movements which might accompany placement of the lesions in the brainstem. Blood pressure was monitored via a femoral arterial catheter and displayed on a Grass polygraph. Lead II of the ECG was also recorded and rectal temperature was maintained between 36 and 38°C with a heat lamp.

Baroreceptor denervation. Baroreceptor nerves were isolated in the neck as previously reported from this laboratory (4). Baroreceptor denervation was accomplished by bilateral section of the carotid sinus, aortic depressor, and vagus nerves.

Brainstem lesions and histology. Animals were placed in a David Kopf Instruments stereotaxic apparatus after which the dorsal aspect of the medulla was exposed by removal of a portion of the occipital bone and cerebellum. Medial medullary lesions were made with an electrode 0.5 mm in diameter whose tip was exposed 7 mm so that the lesion would extend from the dorsal to the ventral surface of the brainstem. Lesions were made in the midline 0–2 mm rostral to the obex in several steps by passing 5 mA of anodal current for 15 sec. The cathode was an electrode attached to the temporal muscle. Electrolytic lesions (5 mA for 15 sec) of the nucleus of the tractus solitarius (NTS) were made bilaterally 1 mm rostral and 1 mm caudal to the obex. As described by Miura and Reis (5), the posterointermediate sulcus was used as a surface landmark for localization of NTS along its rostral–caudal extent. The electrode was placed on or just medial to the sulcus and 1.3–1.5 mm below the surface of the medulla.

The extent of the electrolytic lesions of medial medullary structures and of NTS was

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determined by examination of histological sections. The medulla was removed and fixed in 10% buffered formalin following each experiment. Frontal sections of 30 μm thickness were cut with a cryostat microtome and stained with cresyl violet.

Decerebration. Pretentorial decerebration was performed with a spatula placed in a stereotaxic electrode holder. The spatula 8 mm in diameter was lowered first through the right side and then through the left side of the brainstem at the midcollicular level. The completeness of decerebration was visually evaluated at the end of each experiment.

Sympathetic nerve recordings and data analysis. A branch of the left renal postganglionic sympathetic nerve was exposed via a retroperitoneal approach, as previously reported from this laboratory (4). SND was recorded monophasically under oil with bipolar platinum electrodes after capacity-coupled preamplification (low and high half-amplitude responses at 1 and 1000 Hz). The output of the preamplifier was led to a Grass 7P10 cumulative integrator. Raw and integrated SND were displayed on a polygraph. Changes in SND were quantified by comparing the mean epoch length (integrated records) during 5 min periods before and at various times after medullary lesions or baroreceptor denervation.

The phase relations between SND and the cardiac cycle were analyzed by computer summation (Nicolet model 1070). The sweep of the computer was triggered by a timing pulse coincident with the R wave of the ECG. Summed records of SND and the arterial pulse wave were displayed on an oscilloscope (Tektronix model 502) and photographed on 35 mm film.

Statistical analysis. Statistical analysis was performed with the Student t test for paired and unpaired data. P values of <0.05 were considered to indicate statistical significance. Values are expressed as mean ± SE.

Results. Medial medullary lesions. Blood pressure and SND are decreased by electrical stimulation of the paramedian reticular (4), raphe obscurus and raphe pallidus (6) nuclei of the medial medulla. Whether these effects are mediated over baroreceptor internuncial networks remains controversial (5, 7–9). Moreover, it is not known whether sympathoinhibitory elements in this region are tonically active in the baroreceptor denervated cat. With regard to these questions, the effects on renal SND and blood pressure produced by electrolytic lesions of medial medullary structures located 0–2 mm rostral to the obex were compared in baroreceptor intact and denervated cats.

The upper panels (A and B) in Fig. 1 show an example of the effects on renal SND and blood pressure produced by medial medullary lesions in a cat with intact baroreceptor nerve fibers. SND and blood pressure decreased (not shown) during application of anodal current. Approximately 30 min was required after the lesions were made for SND and blood pressure to stabilize. At this time (panel B), the level of renal nerve activity was considerably higher than before (panel A) placement of the lesions. As shown in Table IA, renal SND was increased to 142% of control 30 min after medial medullary lesions in four cats with intact baroreceptor nerves. Although blood pressure was somewhat elevated 30 min after placement of the lesions in the experiment illustrated in Fig. 1, the increase in SND produced by medial medullary lesions was not accompanied by a statistically significant change in arterial pressure (Table IA). No further changes in SND and blood pressure were observed for the duration of the experiment (1–2 hr).

SND normally is synchronized into bursts which are locked in a 1:1 relation to the cardiac cycle by the baroreceptor reflexes (4). The R wave-triggered computer summed records (inset) in panel A of Fig. 1 clearly depict the cardiac-related periodicity in SND. Lesions of the medial medulla which increased SND did not disrupt the phase relations between SND and the cardiac cycle (inset in panel B). Moreover, in three experiments, medial medullary lesions which led to marked increases in basal renal SND had little effect on the inhibition of SND associated with the rise in blood pressure produced by the iv injection of 1 μg/kg of norepinephrine bitartrate (Fig. 2). Gebber *et al.* (10) have previously demonstrated that norepinephrine-induced reflex inhibition of SND is eliminated by bilateral section of the baroreceptor nerves in cats anesthetized with the dial–urethane mixture used in the present study. These observations indicate that medial medullary lesions had little or no effect

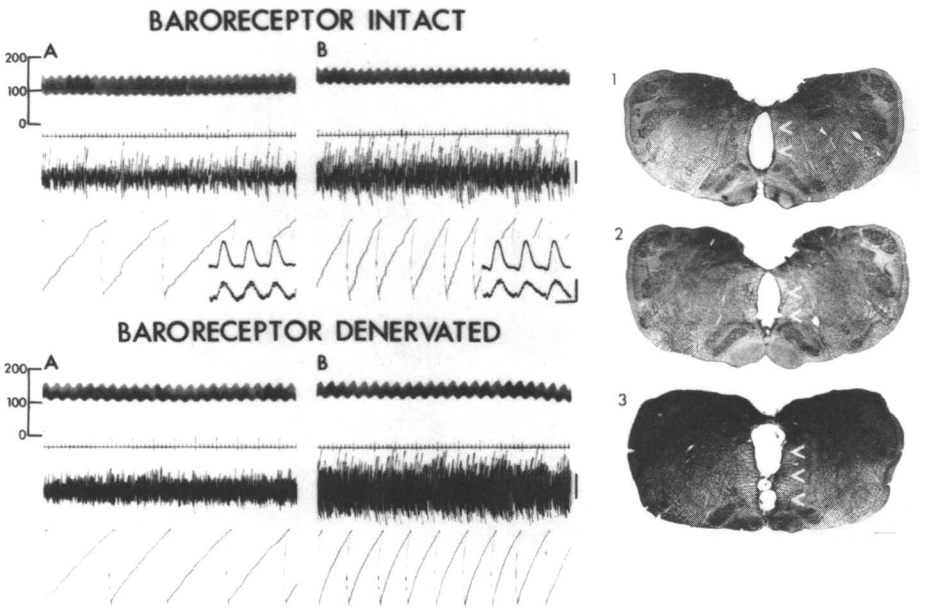

FIG. 1. Effects of medial medullary lesions on blood pressure and renal SND in a baroreceptor intact (upper panels) and a baroreceptor denervated (lower panels) cat. Panels A: before lesions. Panels B: 30 min after lesions. Top trace in each panel is blood pressure (mmHg). Middle trace is SND. Bottom trace shows integrated SND. Time base (below blood pressure) is 1 sec/division. Vertical calibrations for polygraphic traces of SND are 100 μV. Insets in bottom traces in the experiment on baroreceptor intact cat show R wave-triggered computer summed records (64 trials) of arterial pulse wave (top) and SND (bottom). Horizontal calibrations for computer summed records is 250 msec. Vertical calibration is 67 μV and refers to SND. Address bin for computer summed records was 4 msec. Histological sections on the right show the extent of medial medullary lesions in the baroreceptor intact cat. 1: medullary section 2 mm rostral to the obex. 2: 1 mm rostral to the obex. 3: level of the obex. Bar = 1 mm. Arrows point to lesions.

TABLE I. EFFECTS OF MEDULLARY LESIONS AND BARORECEPTOR DENERVATION ON RENAL SND AND BLOOD PRESSURE.

Procedure	SND 30 min after (% of control)	Mean blood pressure Control (mmHg)	30 min after (mmHg)
A) Medial Medullary Lesions			
Baroreceptor intact	142 ± 11 (4)*[a]	123 ± 3	123 ± 7
Baroreceptor denervated[b]	161 ± 13 (11)*	133 ± 5	135 ± 4
Denervated-decerebrated	93 ± 5 (5)	137 ± 5	130 ± 4
B) Baroreceptor Denervation	140 ± 10 (10)*	138 ± 6	144 ± 7
C) NTS Lesions			
Baroreceptor intact	154 ± 13 (4)*	137 ± 8	143 ± 12
Baroreceptor denervated	109 ± 12 (5)	130 ± 11	131 ± 15

[a] Mean ± S.E. (n).
[b] Bilateral section of the carotid sinus, aortic depressor, and vagus nerves.
* $P < 0.05$ compared to control (paired comparisons).

on the sympathoinhibitory component of the baroreceptor reflexes. Thus, the increase in SND produced by the lesions presumably was due to the release of tonic sympathoinhibition of nonbaroreceptor origin. This contention is supported by the results obtained with medial medullary lesions in cats in which the carotid sinus, aortic depressor, and vagus nerves were bilaterally sectioned. As shown in Table IA, renal SND was increased to 161% of control 30 min after lesions were made in 11 baroreceptor denervated cats. This change was not significantly different from that produced by lesions in baroreceptor

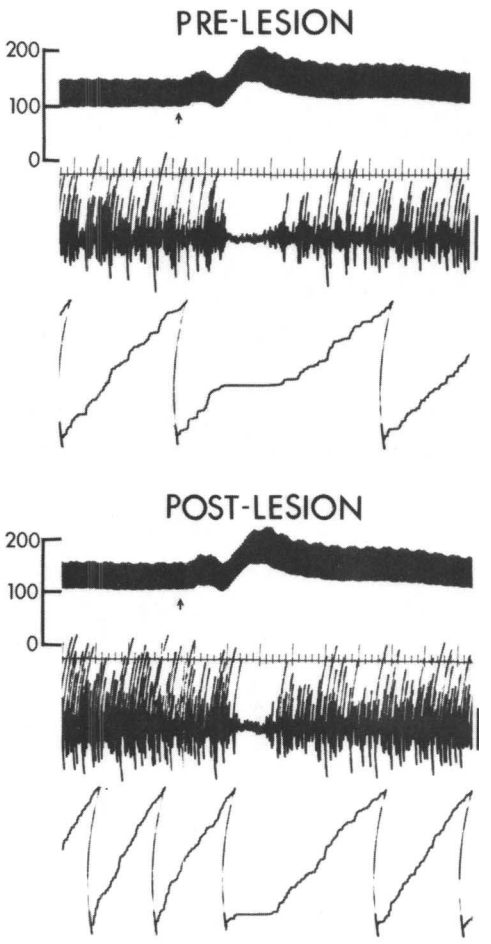

FIG. 2. Reflex inhibition of renal SND associated with rise in blood pressure produced by iv norepinephrine (1 μg/kg) before (upper panel) and 30 min after (lower panel) medial medullary lesions. Sequence of traces is the same as in Fig. 1. Arrows indicate injection of norepinephrine. Time base is 1 sec/division. Vertical calibration is 100 μV and refers to SND.

intact cats. As was the case in baroreceptor intact cats, the increase in SND observed 30 min after placement of the lesions was not accompanied by a change in blood pressure. The effect on SND produced by medial medullary lesions in one of the experiments performed on baroreceptor denervated cats is shown in the lower panels (A and B) in Fig. 1.

The extent of the medial medullary lesions made in baroreceptor intact and denervated cats is shown by the histological sections in Fig. 1. The lesions destroyed the caudal portions of the paramedian reticular, raphe obscurus and raphe pallidus nuclei located 0–2 mm rostral to the obex. The lesioned area did not include the medullary nucleus of baroreceptor fiber termination (i.e., NTS).

In order to determine whether sympathoinhibition involving the medial structures of the caudal medulla is dependent upon the integrity of forebrain-medullary connections, the effects on renal SND produced by lesions of the medial medulla were studied in baroreceptor denervated cats which were decerebrated at the midcollicular level. The results from five experiments are summarized in Table IA and a typical experiment is shown in Fig. 3. Medial medullary lesions failed to increase SND or blood pressure in the decerebrated cat (panel B).

Baroreceptor denervation. The foregoing data suggest that tonic sympathoinhibition involving medial structures of the caudal medulla is of nonbaroreceptor origin. It was of interest to ascertain the relative contributions made by this system and by the baroreceptor reflexes in the control of SND in the anesthetized cat. For this purpose, the effect of bilateral section of the carotid sinus, aortic depressor and vagus nerves on renal SND was studied in 10 cats. The results of these experiments were compared with those from cats in which the medial medulla was lesioned. As shown in Table IB, renal SND was increased to 140% of control 30 min after baroreceptor nerve section. This change was not significantly different from that produced by medial medullary lesions in cats with intact or with sectioned baroreceptor nerves (Table IA). Thus, the baroreceptor and nonbaroreceptor sympathoinhibitory systems were equally important in controlling the level of basal SND under the conditions of our experiments.

A typical experiment depicting the effects of baroreceptor nerve section on renal SND and blood pressure is shown in Fig. 4. Blood pressure and SND were markedly increased 5 min after bilateral section of the carotid sinus, aortic depressor, and vagus nerves (middle panel). As reported by others (1, 11–13), the hypertensive response to baroreceptor nerve section is not sustained, especially in anesthetized preparations. Although SND remained elevated for the duration of the experiment, blood pressure returned near to control level within 30 min after barore-

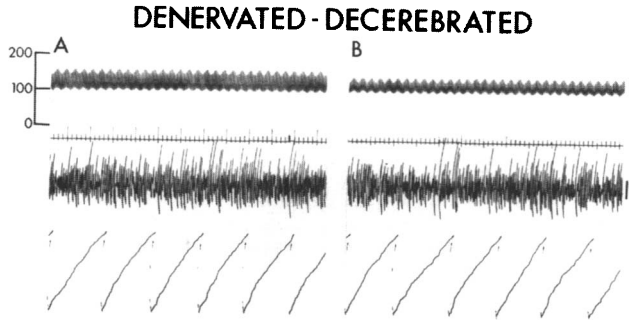

FIG. 3. Effects of medial medullary lesions on blood pressure and renal SND in baroreceptor denervated and decerebrated cat. Sequence of traces is the same as in Fig. 1. A: before lesions. B: 30 min after lesions. Time base is 1 sec/division. Vertical calibration is 100 μV.

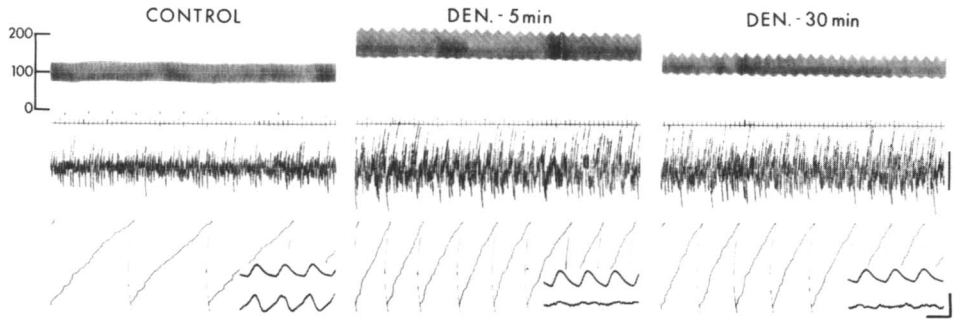

FIG. 4. Effects of baroreceptor denervation on blood pressure and renal SND. Sequence of traces is same as in Fig. 1. Left: control. Middle: 5 min after bilateral section of the carotid sinus, aortic depressor, and vagus nerves. Right: 30 min after baroreceptor denervation. Time base is 1 sec/division and vertical calibration is 100 μV for polygraphic records of SND. Insets in bottom traces show R wave-triggered computer summed records (64 trials) of arterial pulse wave (top) and SND (bottom). Address bin and calibrations for computer summed records are same as in Fig. 1.

ceptor nerve section (Fig. 4, right panel and Table IB). Baroreceptor denervation was complete in these experiments since as shown in the insets in Fig. 4, bilateral section of the carotid sinus, aortic depressor and vagus nerves disrupted the phase relations between SND and the cardiac cycle. The computer summed records of SND approached a straight line after section of these nerves since activity in the renal nerve was no longer locked in time to the R wave trigger by cardiac-related baroreceptor nerve discharge. In addition, as reported previously by Gebber *et al.* (10), section of the aforementioned nerves prevented the reflex inhibition of SND associated with the rise in blood pressure produced by the iv injection of norepinephrine (1–2 μg/kg).

NTS lesions. The intermediate one-third of NTS is the primary site of termination of baroreceptor fibers of the carotid sinus, aortic depressor, and vagus nerves in the cat (7–8, 14–15). Recently, it has been established that some interneurons in NTS receive driving inputs from nonbaroreceptor as well as from baroreceptor afferents. Specifically, McCall *et al.* (16) found that NTS neurons in the baroreceptor reflex arc could be activated by electrical stimulation of afferents in the inferior cardiac nerve. With regard to these findings, it was of interest to determine whether sympathoinhibitory interneurons in the baroreceptor reflex arc remain tonically active after section of the carotid sinus, aortic depressor, and vagus nerves. For this purpose, the effects of NTS lesions on renal SND were compared in baroreceptor intact and denervated cats. The extent of the lesions placed in

NTS 1 mm caudal and 1 mm rostral to the obex is illustrated in the histological sections in Fig. 5. As shown in Table IC and in the upper panels (A and B) of Fig. 5, ablation of the intermediate one-third of NTS significantly increased renal SND in cats in which the baroreceptor nerves were intact. These lesions completely disrupted the phase relations between SND and the cardiac cycle (see insets), thus indicating that the sympathoinhibitory component of the baroreceptor reflexes was eliminated. As was the case with medial medullary lesions or with section of the baroreceptor nerves, blood pressure was not significantly changed 30 min after NTS was lesioned. As shown in Table IC and in the lower panels (A and B) of Fig. 5, NTS lesions failed to alter SND in the baroreceptor denervated cat. This observation indicates that sympathoinhibitory interneurons in NTS are not tonically active in the absence of baroreceptor nerve discharge.

Discussion. The present study has shown that tonic sympathoinhibition of nonbaroreceptor origin is mediated by neuronal elements which originate or pass through medial structures in the caudal medulla of the cat. These structures include portions of the paramedian reticular, raphe obscurus and raphe pallidus nuclei. Our conclusion is based on the observation that medial medullary lesions placed 0–2 mm rostral to the obex produced a significant increase in renal SND in the baroreceptor denervated cat. Lesions of this region in baroreceptor intact cats also increased renal SND without disrupting the phase relations between SND and the cardiac cycle or reflex inhibition of SND associated with the pressor action of norepinephrine. In this regard, section of the baroreceptor nerves completely disrupted the phase relations between SND and the cardiac cycle (Fig. 4) and eliminated reflex inhibition of SND produced by increasing blood pressure with norepi-

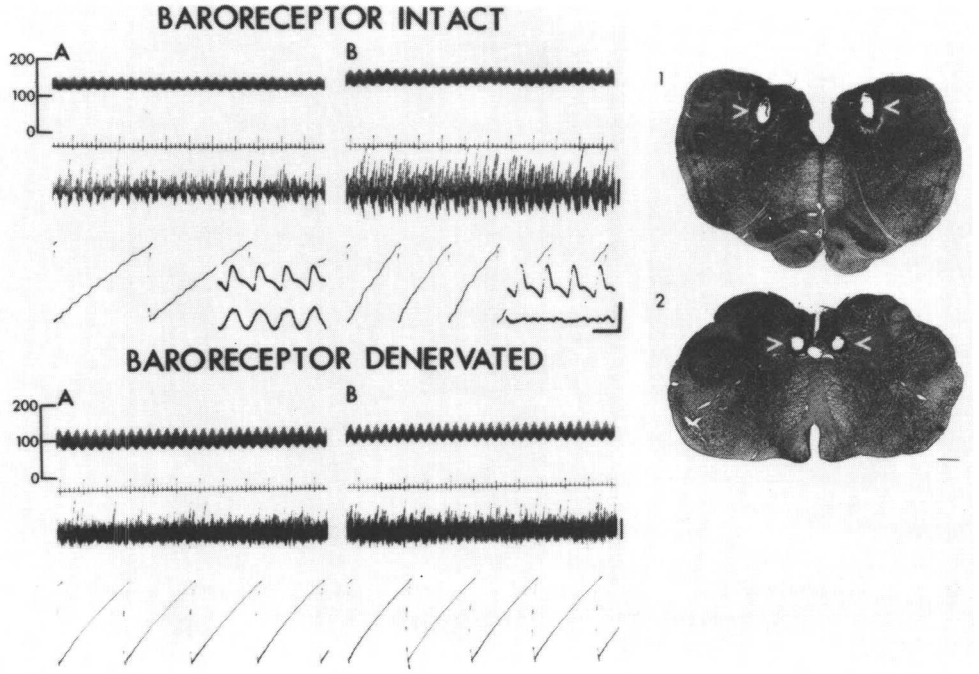

FIG. 5. Effects of NTS lesions on blood pressure and renal SND in a baroreceptor intact (upper panels) and a baroreceptor denervated (lower panels) cat. Sequence of traces in each panel is same as in Fig. 1. Panels A: before lesions. Panels B: 30 min after lesions. Time base is 1 sec/division and vertical calibration for polygraphic traces of SND is 100 μV. Insets in bottom traces in the experiment on the baroreceptor intact cat show R wave-triggered computer summed traces of arterial pulse wave (top) and SND (bottom). Address bin and calibrations for computer summed records are same as in Fig. 1. Histological sections on the right show extent of NTS lesions in the baroreceptor intact cat. 1: 1 mm rostral to the obex. 2: 1 mm caudal to the obex. Bar = 1 mm. Arrows point to lesions.

nephrine. Thus, it would appear that tonic sympathoinhibition from the medial medulla is transmitted through a brainstem circuit distinct from that responsible for baroreceptor reflex effects on SND.

The increase in SND produced by medial medullary lesions in baroreceptor denervated cats was prevented by prior decerebration. This observation argues against the possibility that the increase in SND produced by lesions of the medial medulla was due to irritation of surrounding sympathoexcitatory regions rather than to the release of neural inhibition. It is also clear from this result that tonic sympathoinhibition of nonbaroreceptor origin is somehow dependent upon the integrity of forebrain–medullary connections. It is possible that tonic sympathoinhibition involving midline structures of the caudal medulla is of forebrain origin. In this regard, Lofving (17) demonstrated that the decrease in blood pressure produced by electrical stimulation of the anterior hypothalamus was prevented by ablation of the medial medulla. Alternatively, the inability to produce an increase in SND with medial medullary lesions in the decerebrated cat might indicate that tonic sympathoinhibition of nonbaroreceptor origin acts specifically to suppress SND emanating from the forebrain. Concerning this possibility, it has been claimed (18) that sympathoexcitatory elements in the posterior hypothalamus are tonically active in the anesthetized cat.

The manner in which activity in the nonbaroreceptor sympathoinhibitory system is generated remains obscure. Nevertheless, it is noteworthy that the increase in renal SND produced by medial medullary lesions in cats with an intact neuraxis was not significantly different from that observed following section of the baroreceptor nerves or after lesions of the medullary nucleus of baroreceptor fiber termination (i.e., NTS). This observation suggests that the nonbaroreceptor sympathoinhibitory system is potentially as important as the baroreceptor reflexes in the control of SND in the anesthetized cat.

The increase in SND produced by medial medullary lesions was not accompanied by an increase in blood pressure. This observation undoubtedly explains why such lesions increased SND to the same extent in baroreceptor intact and denervated cats. That is, the expected increase in baroreceptor reflex activity which might have compensated for the removal of sympathoinhibition of nonbaroreceptor origin did not occur in cats with intact carotid sinus, aortic depressor, and vagus nerves since blood pressure was not changed by medial medullary lesions. At first glance it is disconcerting that blood pressure failed to rise in the face of elevated SND following medial medullary lesions. However, the observation is not so surprising in view of the results obtained with baroreceptor denervation. As noted by others (1, 11–13), the rise in blood pressure immediately following section of the baroreceptor nerves was not sustained beyond 30 min, a period equivalent to that required for SND to stabilize after placement of medullary lesions. In contrast, the increase in SND produced by baroreceptor denervation persisted for the duration of the experiment. Thus, it is apparent that in the anesthetized cat, powerful nonneural mechanisms act to compensate for changes in blood pressure associated with increased SND. It has been suggested that the rapid dissipation of the hypertensive response produced by baroreceptor nerve section might involve a decrease in cardiac output (11) and/or "whole body circulatory autoregulation" (19). These or other nonneural mechanisms might also have precluded us from viewing significant increases in blood pressure following medullary lesions which increased SND.

Finally, the present study has demonstrated that lesions of NTS increased SND only in cats in which the baroreceptor nerves were intact. This observation indicates that sympathoinhibitory elements within the medullary nucleus of baroreceptor fiber termination are not tonically active in the anesthetized cat after section of the carotid sinus, aortic depressor, and vagus nerves. Thus, auxiliary afferent input to baroreceptor interneurons in NTS (16) are insufficient to bring these cells to discharge threshold in the absence of baroreceptor nerve activity.

Summary. Lesions of those portions of the paramedian reticular, raphe obscurus and raphe pallidus nuclei which extend 0–2 mm rostral to the obex increased renal SND without affecting the baroreceptor reflexes in cats with intact carotid sinus, aortic depressor, and vagus nerves. Lesions of these medial

medullary structures in baroreceptor denervated cats produced an equivalent increase in SND. The effect of medial medullary lesions on SND, however, was prevented by prior decerebration. These results indicate that tonic sympathoinhibition involving the medial medulla is of nonbaroreceptor origin and is dependent upon the integrity of forebrain–medullary connections. The increase in SND produced by medial medullary lesions was not significantly different from that produced by section of the baroreceptor nerves or by ablation of the medullary nucleus of baroreceptor fiber termination (i.e., NTS). Thus, the nonbaroreceptor sympathoinhibitory system of the medial medulla is potentially as important as the baroreceptor reflexes in the control of SND in the anesthetized cat. Finally, this study also demonstrated that sympathoinhibitory elements in NTS are not tonically active in the absence of baroreceptor nerve input.

1. Heymans, C., and Neil, E., "Reflexogenic Areas of the Cardiovascular System," pp. 271 Little, Brown and Company, Boston (1958).
2. Alexander, R. S., J. Neurophysiol. **9,** 205 (1946).
3. Coote, J. H., Downman, C. B. B., and Weber, W. V., J. Physiol. (London) **202,** 147 (1969).
4. Taylor, D. G., and Gebber, G. L., Amer. J. Physiol. **228,** 1002 (1975).
5. Miura, M., and Reis, D. J., J. Physiol. (London) **223,** 525 (1972).
6. Coote, J. H., and Macleod, V. H., J. Physiol. (London) **241,** 477 (1974).
7. Crill, W. E., and Reis, D. J., Amer. J. Physiol. **214,** 269 (1968).
8. Humphrey, D. R., *in* "Baroreceptors and Hypertension" (P. Kezdi, ed.) p. 131, Pergamon Press, New York (1967).
9. Spyer, K. M., and Wolstencroft, J. H., Brain Res. **26,** 411 (1971).
10. Gebber, G. L., Taylor, D. G., and Weaver, L. C., Amer. J. Physiol. **224,** 470 (1973).
11. Charlier, R., and Phillipot, E., Arch. Internat. Physiol. **55,** 170 (1947).
12. Cowley, A. W., Liard, J. F., and Guyton, A. C., Circ. Res. **32,** 564 (1973).
13. Strait, M. R., and Brody, M. J., Fed. Proc. **36,** 547 (1977).
14. Cottle, M. K., J. Comp. Neurol. **122,** 329 (1964).
15. Seller, H., and Illert, M., Pflügers Arch. **306,** 1 (1969).
16. McCall, R. B., Gebber, G. L., and Barman, S. M., Amer. J. Physiol. **232,** H657 (1977).
17. Lofving, B., Acta Physiol. Scand. **53,** Suppl. 184, 1 (1961).
18. Gellhorn, E., Amer. Heart J. **67,** 106 (1964).
19. Coleman, T. G., Granger, H. J., and Guyton, A. C., Circ. Res. **28** and **29,** Suppl. II, II-76 (1971).

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