Participation of Central Noradrenergic Neurons in Arterial Baroreceptor Reflexes in the Rabbit

By J. P. Chalmers and R. J. Wurtman

ABSTRACT

Disappearance rates of intracisternally administered $^3$H-norepinephrine and activities of tyrosine hydroxylase were examined in the rabbit in five brain regions (telencephalon, hypothalamus, midbrain, medulla-pons, and cerebellum) and in three cord regions (cervical, thoracolumbar, and lumbosacral) 2 weeks after section of the carotid sinus and aortic nerves. Mean blood pressure rose by 29% and heart rate by 17% in the animals with neurogenic hypertension. Endogenous catecholamine concentrations in the eight regions examined were not altered by denervation. In the thoracolumbar region of the spinal cord, $^3$H-norepinephrine turnover and tyrosine hydroxylase activity were increased approximately twofold in hypertensive rabbits. We suggest that these changes reflect increased physiological activity of bulbospinal noradrenergic neurons and that this increase may mediate the rise in arterial pressure or heart rate that follows sinoaortic denervation. The turnover of $^3$H-norepinephrine increased in the hypothalamus of denervated animals; tyrosine hydroxylase activity remained unchanged in this region.

KEY WORDS: neurogenic hypertension, mean blood pressure, sympathetic nervous system, thoracolumbar spinal cord, catecholamine, tyrosine hydroxylase activity, carotid sinus, hypothalamus, heart rate

Denervation of the carotid sinus and aortic arch baroreceptors eliminates a major source of inhibition of tonic peripheral sympathetic activity and produces a neurogenic hypertension accompanied by tachycardia (1-4). The central neuroanatomical pathways of baroreceptor reflexes have been broadly identified (2, 5, 6), but little is known about the neurotransmitters utilized by the neurons involved.

A growing body of evidence suggests that norepinephrine functions as a neurotransmit-
organization of central adrenergic neurons is thus compatible with the possibility that baroreceptor reflexes are mediated at least in part by nonadrenergic pathways within the brain and spinal cord. In particular, since the neurogenic hypertension produced by section of the buffer nerves depends on increased activity in postganglionic sympathetic vasoconstrictor neurons (13, 14), it seems possible that baroreceptor reflexes might utilize descending adrenergic pathways that ultimately terminate on preganglionic neurons in the lateral horn of the spinal cord.

In the present study, in rabbits with chronic section of the carotid sinus and aortic nerves, this hypothesis is tested by examination of the turnover of intracisternally administered 3H-norepinephrine and the activity of tyrosine hydroxylase, the rate-limiting enzyme in catecholamine biosynthesis, in appropriate regions of the brain and spinal cord.

**Methods**

**SECTION OF CAROTID SINUS AND AORTIC NERVES**

New Zealand white male rabbits weighing 2 to 3 kg were fed on Big Red Rabbit Food pellets (Country Best Foods, Agway, Inc., Waverly, New York) and water ad libitum. The rabbits were anesthetized with sodium pentobarbital (30 to 40 mg/kg iv) and were placed on their backs with necks extended. Lidocaine (0.5%) was infiltrated subcutaneously in the midline from the jaw to the thoracic inlet, the skin was incised, the strap muscles were separated, and the carotid arteries were exposed. The aortic nerves were cut just below their junction with the superior laryngeal nerves, and a 3-cm segment of each nerve was excised. These nerves carry fibers from baroreceptors in both common carotid arteries as well as from the aortic arch (left side) and the origin of the right subclavian artery (right side) (1, 3), and in the rabbit they are completely separate from both the vagus nerve (left side) and the cervical sympathetic trunk. The carotid bifurcation was then exposed on each side, and the adventitia of the external, internal, and common carotid arteries was stripped for 1 cm from the bifurcation. Sham-operated animals were anesthetized and their carotid and aortic nerves were exposed but not touched. At the end of the operation all animals received 100,000 units of penicillin im. The completeness of the procedure for denervating the arterial baroreceptors was confirmed in a subgroup of animals by demonstrating that bradycardia did not occur in response to the rise in blood pressure produced by injecting 10 μg of norepinephrine into the right atrium (4, 13).

**ARTERIAL PRESSURE AND HEART RATE**

Unanesthetized rabbits were placed in a standard rabbit box and the central ear artery was cannulated after local infiltration anesthesia of the skin of the ear. The catheter consisted of a 3-mm tip of fine polyethylene tubing (50 gauge) glued inside a 30-mm length of larger polyethylene tubing (100 gauge) and was kept filled with heparinized 0.85% saline. The animals were then allowed to rest quietly in the rabbit box for 1 hour before recording was begun. The noise level in the laboratory was kept low, and the animals were not handled during recording. Mean ear artery pressures were recorded with a Statham P23 Db strain gauge connected to a Hewlett Packard carrier preamplifier (8805A) and a Hewlett Packard Model 7703H recorder. The values reported for arterial pressure and heart rate represent the mean of four determinations taken at 10-minute intervals. The heart rate was obtained by counting from the arterial pressure record. Arterial pressures and heart rates were recorded 1 or 2 days before denervation or sham operation in some animals and 14 to 16 days postoperatively in all animals.

**INTRACISTERNAL INJECTION OF 3H-NOREPINEPHRINE**

1, 2, 3-H-norepinephrine (specific activity, 7.9 c/mm, New England Nuclear Corp., Boston, Mass.) was used for intracisternal injection. It was first purified by passage over a cation exchange column (Dowex 50W-X2, 200-400 mesh, H+) and then over a reactivated alumina column. The injection solution was made isotonic with cerebrospinal fluid and adjusted to pH 6.5 just before use.

Rabbits were lightly anesthetized, as described above, and after the hair at the back of the neck had been clipped were laid prone with neck extended. The head was lifted up and the neck gently flexed. 3H-norepinephrine (13.8 μc) was injected through the skin into the cisterna magna in a volume of 100 μliters with a 26-gauge, half-inch, stainless steel hypodermic needle attached to a graduated Hamilton syringe (15). Appropriate distribution and turnover studies have confirmed that intracisternally injected 3H-norepinephrine mixes with endogenous norepinephrine stores in the brain and spinal cord of the rabbit and provides a suitable tracer for investigation of norepinephrine metabolism (15), as has
been documented previously for the rat (16-19).

After the intracisternal injection, the animals were maintained in the prone position during recovery from anesthesia (about 1 hour) and were then put back into their cages, where they appeared to behave, eat, and drink quite normally. They were killed at various times after the injection by an overdose of sodium pentobarbital.

**DISSECTION PROCEDURE**

The brain and spinal cord were immediately removed, rinsed with water, blotted, and dissected over ice. The brain was dissected into 5 parts: medulla oblongata plus pons (rhombencephalon or medulla-pons), cerebellum, midbrain, hypothalamus, and the remaining portion of the forebrain, composed of the thalamus and the cerebral hemispheres (telencephalon). The spinal cord was divided into three segments: cervical, thoracolumbar (T1-L3), selected to coincide with the portion of the cord having a lateral horn and giving rise to the peripheral sympathetic division of the autonomic nervous system), and lumbo-sacral. The dissection procedure was based on descriptions of the rabbit brain in the stereotaxic atlas of Monnier and Gangloff (20). The removal and dissection of brain and spinal cord took approximately 30 minutes. Most tissues were immediately frozen on dry ice, weighed, and stored at −20°C until they were assayed; those used for tyrosine hydroxylase assay were weighed without freezing and were homogenized immediately.

**ASSAYS**

Catecholamines.—Tissues were homogenized in 5 volumes of 0.4M perchloric acid and centrifuged at 17,000 x g for 10 minutes. The supernatant was poured over alumina columns at pH 8.6 (21). The acetic acid eluates from these columns were used for estimation of 3H-norepinephrine by liquid scintillation spectrophotometry (22) and of nonradioactive norepinephrine and dopamine by spectrophotofluorometry (23, 24). The values for tissue levels of 3H-norepinephrine at 6, 9, and 12 hours after intracisternal injection were logarithmically transformed (to the base 10) for calculation of the regression coefficient (slope), and analysis of variance (25) was used for calculation of the standard error of the regression coefficient, the significance of each individual regression coefficient, and the significance of the difference between regression coefficients. The half-lives for the rates of 3H-norepinephrine disappearance during this period were derived by the formula $T_1/2 = \log_{10}2/\text{regression coefficient}$.

Tyrosine Hydroxylase.—The animals used for this experiment were killed in a cold room at 4°C, and all subsequent maneuvers prior to incubation were carried out in the cold room or in a refrigerated centrifuge. Tissues were homogenized in ice-cold distilled water, and the enzyme tyrosine hydroxylase was partially purified (26). The activity of the enzyme was assayed by measuring the conversion of L, 3, 5-3H-tyrosine (New England Nuclear Corp.) to 3H-dihydroxyphenylalanine (3H-dopa) (27). The contents of the incubation mixture were as described by Musacchio et al. (28). Samples were assayed in duplicate, and a blank was prepared for each sample by addition of an inhibitor, 3-iodo-l-tyrosine. In a typical assay of the thoracolumbar cord, a region with low tyrosine hydroxylase activity, sample incubations yielded 497 and 537 counts/min, whereas the blank tube yielded 249 counts/min.

For all phases of the experiments, denervated and sham-operated animals were alternated within each group, beginning with the initial measurement of arterial pressure and heart rate and maintaining the same order for the intracisternal injection of 3H-norepinephrine, for killing, and for assay of the various tissues.

**Results**

**BLOOD PRESSURE AND HEART RATE IN SHAM-OPERATED AND DENERVATED ANIMALS**

The blood pressure and heart rate were measured 1 to 2 days before and 14 to 16 days after operation in the 20 sham-operated and 20 denervated animals used for the 3H-norepinephrine turnover studies (Table 1). The mean ear artery pressure increased by 29% ($P < 0.001$) and the heart rate by 17% ($P < 0.001$) 2 weeks after section of the carotid sinus and aortic nerves (Table 1). Blood pressure and heart rate were not altered by the sham operation. The changes in arterial pressure and heart rate in the subgroup used for determination of endogenous brain and spinal cord dopamine (Table 1) were similar to those found in the 3H-norepinephrine turnover experiment. In another experiment in which tyrosine hydroxylase activity of brain and spinal cord regions was compared in normal and denervated animals, the blood pressure and heart rate were measured 2 weeks postoperatively (Table 1); the arterial pressure was 34% higher ($P < 0.05$) and the heart rate 23% faster ($P < 0.05$) in the
TABLE 1

<table>
<thead>
<tr>
<th></th>
<th>Sham Operation</th>
<th>Denervation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean arterial pressure (mm Hg)</td>
<td>Heart rate (beats/min)</td>
</tr>
<tr>
<td>Preop.</td>
<td>77.9 ± 1.7</td>
<td>77.5 ± 1.4</td>
</tr>
<tr>
<td>Postop.</td>
<td>82.3 ± 2.5</td>
<td>76.7 ± 3.1</td>
</tr>
</tbody>
</table>

Values are means ± SE; the number of animals is given in parentheses. *Significantly different from preoperative value, \( P < .001 \); †significantly different from sham-operated control, \( P < .05 \); ‡significantly different from sham-operated control, \( P < .02 \). Animals were killed 2 weeks after operation.

denervated group than in the sham-operated group.

ENDOGENOUS CATECHOLAMINE CONCENTRATIONS

Endogenous norepinephrine and dopamine concentrations were measured in the five brain regions and three spinal cord regions listed in Table 2, 2 weeks after sham operation or buffer nerve section. Endogenous norepinephrine was assayed on the same tissues as the \( ^{3} \)H-norepinephrine determinations reported in the disappearance studies. Dopamine values were obtained from the subgroup of rabbits killed 9 hours after the intracisternal injection. There were no significant differences between the endogenous norepinephrine and dopamine concentrations of normal and denervated animals in any of the brain or cord regions examined (Table 2).

TABLE 2

<table>
<thead>
<tr>
<th>Brain region</th>
<th>Endogenous Norepinephrine Concentration (ng/g)</th>
<th>Endogenous Dopamine Concentration (ng/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sham operation</td>
<td>Denervation</td>
</tr>
<tr>
<td>Telencephalon</td>
<td>174 ± 8.9</td>
<td>175 ± 9.2</td>
</tr>
<tr>
<td>Hypothalamus</td>
<td>1280 ± 74</td>
<td>1255 ± 42</td>
</tr>
<tr>
<td>Midbrain</td>
<td>301 ± 12.5</td>
<td>301 ± 7.9</td>
</tr>
<tr>
<td>Brainstem</td>
<td>288 ± 15.8</td>
<td>388 ± 18.8</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>91 ± 5.7</td>
<td>87 ± 4.1</td>
</tr>
<tr>
<td>Spinal cord region</td>
<td>129 ± 6.8</td>
<td>119 ± 4.7</td>
</tr>
<tr>
<td>Thoracolumbar</td>
<td>39 ± 4.6</td>
<td>30 ± 3.8</td>
</tr>
<tr>
<td>Lumbosacral</td>
<td>102 ± 7.4</td>
<td>98 ± 4.9</td>
</tr>
</tbody>
</table>

Values are means ± se. Each is based on 20 determinations (norepinephrine values) or 4 determinations (dopamine values). Animals were killed 2 weeks after operation.
Disappearance of $^{3}$H-norepinephrine from hypothalamus, midbrain, and medulla-pons after intracisternal injection of 12.8 $\mu$g of $^{3}$H-norepinephrine in sham-operated animals and in animals with sinoaortic denervation 2 weeks postoperatively. Each point is the mean of four determinations ± se. Whenever 2 se are shown for each of a pair of corresponding points, the difference between the points is significant at least at the 5% level, where only a single se is indicated for each of two corresponding points (in diverging directions), the difference between the points is not significant ($P > 0.05$).

Disappearance of $^{3}$H-norepinephrine from telencephalon and cerebellum after intracisternal injection of 12.8 $\mu$g of $^{3}$H-norepinephrine 2 weeks postoperatively. Symbols are as in Figure 2.

Injection. Since there were no differences in the endogenous norepinephrine concentrations of corresponding tissues of the two preparations, the data for $^{3}$H-norepinephrine concentration are expressed as ng/g. The decline of $^{3}$H-norepinephrine concentration in all brain and spinal cord regions of both control and denervated animals was multiphasic (Figs. 1-3). In all cases there was a rapid initial disappearance between 10 minutes and 1 hour after injection, followed by a slower single-exponential decay phase during the period from 6 to 12 hours after injection.

The 10-minute accumulation of $^{3}$H-norepinephrine, which provides a measure of uptake (24), was significantly higher in the hypothalamus ($P < 0.001$), midbrain ($P < 0.05$), and thoracolumbar cord ($P < 0.02$) of animals with section of the buffer nerves (Figs. 1 and 3). The $^{3}$H-norepinephrine concentrations at this time in the telencephalon, medulla-pons, cervical cord, and lumbosacral cord of the denervated animals were not significantly different from those in the corresponding regions of control animals; uptake, however,
Disappearance of 3H-norepinephrine from cervical, thoracolumbar, and lumbosacral segments of the spinal cord after intracisternal injection of 13.8 μC of 3H-norepinephrine 2 weeks postoperatively. Symbols are as in Figure 1.

The rate of 3H-norepinephrine disappearance during the first hour after injection was not significantly different in denervated animals from that in sham-operated animals in any of the regions examined (Figs. 1-3). There were highly significant differences, however, between the disappearance rates of the two groups during the major single-exponential decay phase between 6 and 12 hours after injection. In the brain, the disappearance of 3H-norepinephrine was more rapid during this phase in the hypothalamus (Fig. 1) of rabbits with sinoaortic denervation than it was in control rabbits; in the spinal cord, disappearance was more rapid in the thoracolumbar region of the denervated group than in that of the control animals (Fig. 3). The difference between the regression coefficients (slopes) for the disappearance of 3H-norepinephrine (Table 3) in denervated and control animals in those two regions was significant (P<0.02). In the midbrain, as in the hypothalamus, there were significant differences between control and denervated animals in the amounts of 3H-norepinephrine remaining at 9 hours and 12 hours after the intracisternal injection (Fig 1), yet apparent differences in the slopes of decline were not statistically significant (0.05<P<0.1). Differences in the rate of disappearance of 3H-norepinephrine from the lumbosacral cord in the two groups of animals were also not statistically significant (Table 3). In the cerebellum, the slopes for 3H-norepinephrine disappearance were similar in the two groups despite the difference in initial uptake noted earlier (Fig. 2, Table 3).

The half-lives for this major, single-exponential phase of 3H-norepinephrine disappearance are all given in Table 3. The half-lives for the disappearance of 3H-norepinephrine in the three spinal cord regions were very similar in the denervated animals (Table 3, Fig. 4). In normal animals, however, with intact baroreceptor reflexes, the disappearance rate of 3H-norepinephrine was slower in the thoracolumbar segment of the cord than in the cervical cord (P<0.05) or the lumbosacral cord (Table 3, Figs. 3 and 4). The activity of baroreceptor reflexes in intact animals appears to reduce selectively the disappearance rate of 3H-norepinephrine in that segment of the spinal cord that has an intermediolateral cell column and gives rise to sympathetic preganglionic nerve fibers.

Tyrosine hydroxylase activity

Regional tyrosine hydroxylase activity was measured in the central nervous system of six different regions: cerebral cortex, hypothalamus, thalamus, midbrain, pons, and medulla oblongata. The results are expressed as the percentage of the control value. The activity of tyrosine hydroxylase was significantly lower in the cerebellum, brainstem, and spinal cord of control animals than in the cerebellum, brainstem, and spinal cord of denervated animals (P<0.05). These differences suggest that the activity of tyrosine hydroxylase is influenced by the integrity of the sympathetic nervous system.
control and six denervated rabbits 2 weeks after operation (Table 4). The activity of this enzyme increased 95% in the thoracolumbar cord of animals with section of the carotid sinus and aortic nerves ($P < 0.01$) compared to that of sham-operated animals; the activity in the lumbar sacral cord also increased by 42% in denervated animals ($P < 0.01$). No significant differences were observed in the tyrosine hydroxylase activities of other regions of the brain or spinal cord in the denervated animals compared to control rabbits. There was a wide variation in the tyrosine hydroxylase activity of the three spinal cord regions in normal animals, with the lowest activity being in the thoracolumbar segment (Table 4). This variation did not occur in animals with baroreceptor control and six denervated rabbits 2 weeks after operation. Each equation was obtained from 12 pairs of points (4 each at 6, 9, and 12 hours after injection). *$\text{Sa}$* is the standard error of the intercept "a". "$\text{Sb}$" is the standard error of the regression coefficient, "$b$."

All the individual regression coefficients "$b$" were significant at $P < 0.01$ except those for the midbrain and lumbosacral cord of sham-operated animals, where $P > 0.02$.

### Table 4

<table>
<thead>
<tr>
<th>Brain region</th>
<th>Tyrosine Hydroxylase Activity (nmole/g/hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sham-operation</td>
</tr>
<tr>
<td></td>
<td>($\mu g$)</td>
</tr>
<tr>
<td>Telencephalon</td>
<td>4.68 ± 0.01</td>
</tr>
<tr>
<td>Hypothalamus</td>
<td>74.38 ± 12.27</td>
</tr>
<tr>
<td>Midbrain</td>
<td>30.42 ± 3.46</td>
</tr>
<tr>
<td>Medulla-pons</td>
<td>0.14 ± 0.05</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>0.64 ± 0.07</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>Lumbar sacral</td>
<td>1.65 ± 0.16</td>
</tr>
<tr>
<td>Thoracolumbar</td>
<td>0.82 ± 0.12</td>
</tr>
<tr>
<td>Lumbosacral</td>
<td>1.15 ± 0.08</td>
</tr>
</tbody>
</table>

Values are means ± SE and are based on six determinations each. Animals were killed 2 weeks after surgery.

*tyrosine converted to Dopa per hour. †Difference from control is significant, $P < 0.01$. Circulation Research, Vol. XXVIII, April 1971
tor denervation and neurogenic hypertension; in these animals the tyrosine hydroxylase activities of the three regions were similar. The operation of baroreceptor reflexes in normal animals appears to reduce the activity of tyrosine hydroxylase, especially in the thoracolumbar segment of the spinal cord, just as it slows the disappearance of $^3$H-norepinephrine in this segment.

**Discussion**

Norepinephrine in the spinal cord apparently is contained exclusively within the nerve endings of descending noradrenergic neurons (9, 11). The sites of origin of these neurons in the brainstem (8, 9, 11) and the locus of their terminations in the cord (9, 11) (particularly the intermediolateral cell column of the thoracolumbar cord) are compatible with the hypothesis that some of them mediate cardiovascular reflexes.

The present experiments demonstrate that sinoaortic denervation increases the uptake of intracisternally administered $^3$H-norepinephrine in the thoracolumbar segment of the spinal cord and accelerates its rate of disappearance (Fig. 1 and Table 3). The half-life for the decline in $^3$H-norepinephrine concentration in this segment of the cord between 6 and 12 hours after the injection (i.e., the period of single-exponential decay) was less than half that observed in control rabbits (3.5 vs. 9.8 hours). Moreover, the activity of tyrosine hydroxylase, the initial and probably rate-limiting enzyme in catecholamine biosynthesis (27, 28), increased twofold in the thoracolumbar cord during the 2 weeks following section of the buffer nerves. The rates of $^3$H-norepinephrine turnover were very similar in the cervical, thoracolumbar, and lumbosacral regions in denervated animals (Fig. 4, right panel), as were the activities of tyrosine hydroxylase (Table 4). It was only in normal animals, in which descending spinal neurons are subject to the regulatory activity of the arterial baroreceptors, that differences in norepinephrine metabolism were found among these three cord regions (Fig. 4, left panel, and Table 4). The turnover of $^3$H-norepinephrine and the activity of tyrosine hydroxylase were reduced in the thoracolumbar cord of normal animals not only in comparison with the thoracolumbar cord of deservated animals but also in comparison with the other two cord segments in control rabbits (Tables 3 and 4).

It seems likely that the carotid and aortic baroreceptors exert a tonic inhibition on the physiological activity of descending noradrenergic neurons that terminate in the sympathetic lateral horn. This inhibition is reflected in a low rate of $^3$H-norepinephrine turnover and in low tyrosine hydroxylase activity. Abolition of this inhibitory input by section of the carotid sinus and aortic nerves increases the activity of these bulbospinal neurons and leads in turn to neurogenic hypertension or tachycardia or both. The experiments provide the first evidence, to our knowledge, relating the physiological activity of a specific central noradrenergic pathway (i.e., bulbospinal tracts) to the control of a particular physiological function (reflex control of blood pressure or heart rate or both).

Norepinephrine applied electrophoretically to central neurons has been reported to evoke both facilitatory and inhibitory responses (7). Both types of responses can be elicited in the Renshaw cell (29) and in other interneurons (30) in the spinal cord. The increase in the activity of bulbospinal noradrenergic neurons in animals with sinoaortic denervation parallels the increase in activity that occurs in the postganglionic sympathetic neurons in such animals (13, 14). This effect suggests that norepinephrine might be acting as a facilitatory neurotransmitter in the descending spinal pathways involved. It is, of course, equally possible that norepinephrine in the thoracolumbar cord functions as an inhibitory neurotransmitter released by descending inhibitory neurons that do not synapse directly with sympathetic preganglionic cells in the lateral horn but with intervening spinal interneurons.

The assay system employed to measure tyrosine hydroxylase activity in our studies...
uses a partially purified enzyme preparation (26) exposed to optimal in vitro conditions. Hence it seems likely that the increases in cord enzyme activity observed in denervated rabbits reflected actual increases in the amounts of enzyme protein present (26), presumably resulting from removal of a tonic inhibitory input that suppresses the synthesis or accelerates the degradation of tyrosine hydroxylase in descending bulbospinal neurons. The physiological significance of the increase in tyrosine hydroxylase activity within the lumbar-sacral cord (Table 4) of denervated animals is not clear. Minor variations in the anatomical limits of the sympathetic lateral horn, or in the level at which we divided the cords, could have led to inclusion of some lateral horn tissue in the lumbar-sacral segments of the spinal cord.

CHANGES IN THE ACTIVITY OF NORADRENERGIC NEURONS IN THE HYPOTHALAMUS

Norepinephrine in the brain is believed to be contained mainly in neurons having their cell bodies in the brainstem and projecting through the medial forebrain bundle to ramify widely throughout the diencephalon and telencephalon (8, 10, 31). The hypothalamus which is particularly rich in the noradrenergic nerve endings, is known to exert important influences on the regulation of cardiovascular function and to influence the activity of baroreceptor reflexes (2, 5, 6, 12, 32-35).

In the present experiments, an increase was observed in the uptake and rate of disappearance of injected 3H-norepinephrine from the hypothalamus of denervated rabbits with neurogenic hypertension (Fig. 1 and Table 3); there was no accompanying increase in the activity of tyrosine hydroxylase. The increased norepinephrine turnover in the hypothalamus of the denervated rabbits may have reflected increased physiological activity of ascending noradrenergic neurons as a direct consequence of the deafferentation of arterial baroreceptors. It is also possible that the increased turnover in this complex structure was secondary to other physiological changes resulting from sinoaortic denervation.

LIMITATIONS AND ASSUMPTIONS

Methods Used to Assess Physiological Activity of Adrenergic Neurons.—The experimental methods used in the present study have a number of intrinsic limitations, and the interpretation of data derived from their application rests on several assumptions. The activity of adrenergic neurons is not measured directly by neurophysiological techniques but is assessed indirectly from the rate of disappearance of 3H-norepinephrine (36-38) and the activity of tyrosine hydroxylase (14, 26).

There is considerable evidence that intraneuronal norepinephrine is metabolically heterogeneous, existing in multiple dynamic pools (37, 39). This concept is compatible with our observation (Figs. 1-3) that norepinephrine disappears from the brain and cord in a multiphasic fashion. The heterogeneity of intraneuronal norepinephrine may be partly responsible for the imperfect correlation obtained between the regional changes in 3H-norepinephrine disappearance and tyrosine hydroxylase activity observed in denervated animals (Tables 3 and 4).

Radioactive norepinephrine injected into the cerebrospinal fluid is selectively concentrated within adrenergic neurons (16, 19). The uptake sites for this material are heterogeneous, however, in that they include the cell bodies as well as the nerve endings of neurons that take up norepinephrine and dopamine (17, 18). The concentration of dopamine in the spinal cord is very low (Table 2 (40)) and bulbospinal adrenergic pathways are believed to be entirely noradrenergic (8, 9); hence we assume that the observed changes in norepinephrine metabolism in the thoracolumbar cord of denervated animals are occurring in bulbospinal noradrenergic pathways. Differences in the metabolism of norepinephrine in cell bodies compared with nerve endings could possibly underlie the failure to observe changes in the medulla-pons (a region rich in cell bodies) in denervated animals. The techniques used to study norepinephrine turnover and tyrosine hydroxylase activity can only reflect the average changes in activity of...
noradrenergic neurons in any region examined. Since each brain region and possibly each cord region may contain many different populations of noradrenergic neurons subserving different and even opposing functions, it is possible that changes in the activity of some groups were partially or completely masked, for example, in the medulla-pons, midbrain, and lumbar-sacral cord. In these last two regions, (Figs. 3 and 4) the disappearance rates of 3H-norepinephrine appeared different in control and denervated rabbits although the differences did not attain statistical significance (Table 3).

Site of Origin of Increased Activity of Noradrenergic Neurons.—Denervation of the carotid and aortic reflexogenic zones produces neurogenic hypertension by releasing sympathetic vasoconstrictor neurons from the tonic inhibition imposed by the arterial baroreceptors (1, 2). Denervation also produces tachycardia, which may depend on both an increase in sympathetic nerve activity and a reduction in vagal tone (1, 2). The increases in blood pressure and heart rate in our animals 2 weeks after the denervation operation were similar to those described in previous reports on the rabbit (3, 4, 14).

We have suggested that the increased activity of noradrenergic neurons in the thoracolumbar cord and hypothalamus of animals with sinoaortic denervation results from the loss of tonic arterial baroreceptor activity. At least two other possibilities must be considered. The changes could be secondary to loss of tonic arterial chemoreceptor activity after sinoaortic denervation. The effects of arterial chemoreceptor denervation in resting animals breathing room air are much smaller, however, than those of chronic baroreceptor denervation and are limited to a mild respiratory depression without cardiovascular manifestations (1, 4). Furthermore, there is evidence that although mild respiratory depression is detectable in resting animals 2 to 4 days after sinoaortic denervation (4), it becomes imperceptible a few days later (41, 42). It seems unlikely that loss of tonic chemoreceptor activity 3 weeks after section of the carotid sinus and aortic nerves was responsible for the observed changes in activity of central noradrenergic neurons in resting rabbits breathing room air.

The second possibility is that the changes observed in norepinephrine metabolism could have been caused by the elevated blood pressure per se acting directly on the brain and spinal cord or via other receptors. This hypothesis is rendered unlikely by the fact that in rats made hypertensive by administration of DOCA and salt after encapsulation of the left kidney, but having intact arterial baroreceptors, the changes occurring in brain norepinephrine metabolism were the opposite of those reported here, namely, there was a reduction in the rate of disappearance of intrathecally injected 3H-norepinephrine from the hypothalamus of the hypertensive rats (43). Similarly, in spontaneously hypertensive rats with functioning baroreceptors (44) the synthesis of norepinephrine in the brainstem is reduced (45).

These changes are most economically explained by the hypothesis that loss of tonic baroreceptor activity in rabbits with neurogenic hypertension following sinoaortic denervation produces an increase in activity of central noradrenergic neurons mediating cardiovascular reflexes, whereas increased stimulation of baroreceptors in other forms of hypertension leads to a decrease in the activity of central noradrenergic neurons in an attempt to reduce the blood pressure.

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