Participation of Adrenergic and Noradrenergic Neurones in Central Connections of Arterial Baroreceptor Reflexes in the Rat

JOHN P. CHALMERS, MARGARET A. PETTY, AND JOHN L. REID

SUMMARY We examined activities of tyrosine hydroxylase (TH) and phenylethanolamine-N-methyl transferase (PNMT) and concentrations of norepinephrine (NE) in seven brain regions and the spinal cord of Wistar rats with elevated blood pressures 1 week and 4 weeks after denervation of carotid and aortic baroreceptors, and compared them to values in sham-operated control rats. TH activity was increased in the solitary tract nucleus (to 188% of control), parahypoglossal nucleus (to 254%), locus ceruleus (to 191%), and posterior hypothalamus (to 225%) at 1 week but not at 4 weeks after denervation. Similarly, NE concentrations were significantly altered in a number of brain regions at 1 week but not at 4 weeks after denervation. The only change in NE concentration at 4 weeks was in spinal cord where the level was reduced to 80% of control in the denervated rats. In contrast, the only change in PNMT activity 1 week after denervation was in posterior hypothalamus (to 59% of control), whereas at 4 weeks there was an increase in the spinal cord (to 159%) and a decrease in both the anterior and posterior hypothalamus (to 59% and 64% of control, respectively). The experiments suggest that increased activity of noradrenergic neurones in the brain may play a significant role in initiating the increase in pressure produced by baroreceptor denervation, whereas decreased activity of hypothalamic adrenergic neurones may be more important in maintaining the raised pressure. An increase in the activity of both adrenergic and noradrenergic nerves in the spinal cord, however, could contribute to maintaining the increase in blood pressure 4 weeks after denervation.

DEAFFERENTATION of the carotid sinus and aortic arch baroreceptors removes a major inhibitory input into the central nervous system (CNS) and produces an increase in peripheral sympathetic activity (Heymans and Neil, 1958; De Quattro et al., 1969; Korner, 1971; Chalmers, 1975). This results in an increase in the lability of heart rate and blood pressure when measured by telemetry without handling the animals, under resting basal conditions (Cowley et al., 1973), and a long-lasting increase in the level of heart rate and blood pressure (neurogenic hypertension) when measured using methods requiring the handling of the animals under resting laboratory conditions (Heymans and Neil, 1958; De Quattro et al., 1969; Uvnas, 1960; Korner, 1965; Chalmers et al., 1967; Krieger, 1964).

Section of the buffer nerves in the rabbit has been shown to increase the turnover of norepinephrine and the activity of tyrosine hydroxylase in the hypothalamus and spinal cord (Chalmers and Wurtman, 1971). The administration of 6-hydroxydopamine (6-OHDA) into the cerebrospinal fluid both prevents and abolishes the neurogenic hypertension produced by peripheral section of the buffer nerves (Chalmers and Reid, 1972) and by central deafferentation of baroreflexes (Doba and Reis, 1974). Reis and Doba (Doba and Reis, 1973) have been able to produce a marked increase in blood pressure following complete central deafferentation of baroreflexes by destruction of the nucleus tractus solitarius (NTS), the site of the first synapse of the primary sensory neurone subserving baroreceptor afferent function (Palkovits and Zaborsky, 1977). However, selective ablation of the catecholaminergic nerve connections of the NTS produces an increase in the lability of arterial pressure without increasing the mean level of pressure (Reis et al., 1976, 1977).

These experiments clearly demonstrate the importance of central catecholaminergic neurones in arterial baroreceptor reflexes and experimental neurogenic hypertension. They do not permit a distinction between the involvement of norepinephrine-containing neurones and central epinephrine-containing neurones, whose existence did not become apparent until 1974 (Hokfelt et al., 1974). Sensitive assays are now available to measure catecholamines and their biosynthetic enzymes, tyrosine hydroxylase, and phenylethanolamine-N-methyl transferase in various brain regions and the spinal cord.
ase (TH) and phenylethanolamine-N-methyl transferase (PNMT), in individual nuclei in the CNS; these assays have been applied to demonstrate changes in the activity of catecholamine-containing neurones in the spontaneously hypertensive rat, the deoxycorticosterone acetate (DOCA)-salt rat, and the "one-kidney" rat with renovascular hypertension (Saavedra et al., 1976; Versteeg et al.; 1977, Petty and Reid, 1977, 1979). In the present experiments, we have used these assays in the rat with neurogenic hypertension produced by peripheral baroreceptor denervation (Krieger, 1964) to study the role of central epinephrine- and norepinephrine-containing neurones in the central connections of arterial baroreceptor reflexes.

Methods

Denervation of Carotid Sinus and Aortic Baroreceptors

Male Wistar rats (Anglia Laboratories) weighing 200-500 g were anesthetized with chloral hydrate (250-300 mg/kg, ip), and lidocaine (0.5%) was infiltrated subcutaneously in the midline of the neck. The carotid sinus and aortic baroreceptors were denervated bilaterally through a midline incision, using a procedure based on the technique described by Krieger (1964). After identification of the carotid bifurcation, the superior cervical sympathetic ganglion readily was found deep to and closely apposed to the internal carotid artery. A 5-mm segment of the cervical sympathetic trunk then was cut out and removed, immediately caudal to the superior cervical ganglion. A 5-mm segment of the superior laryngeal nerve also was cut out and removed from the redundant loop that crossed the sympathetic trunk at this point, to join the nodose ganglion of the vagus. The common carotid, internal carotid, and external carotid arteries then were thoroughly cleared of all connective tissue and painted with 10% phenol and alcohol for a distance of 5 mm from the bifurcation. The survival of the rats was critically dependent upon the depth of anesthesia, as previously reported (Thant et al., 1969). In the present experiments, the chloral hydrate dosage was sufficient to produce light surgical anesthesia, and under these conditions there were no operative deaths. The efficacy of the denervation procedure was confirmed by the absence of bradycardia following a 30-50 mm Hg increase in pressure induced by intravenous administration of phenylephrine

Sham operations were carried out on the same day on littermates of the same weight using the same procedure of general and local anesthesia; after a midline incision at the front of the neck, the neurovascular sheath was visualized on both sides of the neck, but no attempt was made to dissect out the carotid bifurcation or any of the baroreceptor afferent nerves.

Measurement of Arterial Blood Pressure and Heart Rate

Systolic blood pressure was measured indirectly with a pulse transducer applied to the tail, using a programmed electrotyphgmomanometer (Narco Biosystems) and Grass Polygraph recorder, model RPS 111B (Grass Instrument Co.). Heart rate was obtained by counting from the arterial pressure record.

Dissection Procedure

The rats were killed by decapitation 1 week (acute group) and 4 weeks (chronic group) after operation, and the brains and spinal cords were rapidly removed and dissected over ice. The brain was cut into two portions, using a transverse section immediately caudal to the hypothalamus as visualized on the ventral surface. The hypothalamus was dissected from the rostral portion using the optic chiasm and anterior commissure to delineate it anteriorly and the corpus callosum dorsally. It then was divided into two equal parts which were frozen over dry ice: one the "anterior hypothalamus" and the other the "posterior hypothalamus." The caudal portion of the brain, which comprised the brain stem and cerebellum, was immediately frozen with dry ice and mounted on a stainless steel chuck. Brain stem nuclei and areas of cerebellar cortex were removed from 300-μm-thick frozen sections with special stainless steel needles, according to the microdissection method of Palkovits (1973). The thoracolumbar segments of the spinal cord were removed from the spinal canal and frozen after the cervical and sacral parts were discarded.

Assays

Separate groups of rats were used for assay of norepinephrine, TH, and PNMT. For norepinephrine assay, the tissues were homogenized in 50 μl of 0.1 m perchloric acid (brainstem nuclei and cerebellar cortex) or 10 volumes of this solution (hypothalamus and spinal cord), and 10-μl aliquots were removed for protein estimation according to the method of Lowry et al. (1951). After centrifugation at 4°C (10 minutes at 2000 rpm for brainstem nuclei and cerebellar cortex, and 15 minutes at 10,000 rpm for spinal cord and hypothalamus), norepinephrine was assayed in aliquots of supernatant by the radioenzymatic method of Henry et al. (1975).

To assay TH in brainstem nuclei and cerebellar cortex, tissue samples from two rats were pooled and homogenized in 75 μl of ice cold 0.5 m Tris-HCl buffer, pH 6, containing 0.2% Triton X-100 in a 0.1-ml microhomogenizer (Jencons); anterior and posterior hypothalamus and spinal cord were weighed and homogenized in 10 volumes of this solution. After removal of 3 μl for protein estimation, the homogenates were centrifuged as described above. TH activity was measured in a 40-μl aliquot of
TABLE 1  Concentration of NE and Activity of TH and PNMT in Sham-Operated Rats 1 and 4 Weeks after Operation

<table>
<thead>
<tr>
<th></th>
<th>Concentration of NE (ng/mg protein)</th>
<th>TH activity (nmoles H C-DOPA/mg protein per hr)</th>
<th>PNMT activity (pmole 3H-N-methyl phenylethanolamine/mg protein per hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 wk</td>
<td>4 wks</td>
<td>1 wk</td>
</tr>
<tr>
<td>Nucleus of solitary tract</td>
<td>5.5 ± 1.1</td>
<td>2.8 ± 0.2</td>
<td>21.4 ± 4.8</td>
</tr>
<tr>
<td>Lateral reticular nucleus</td>
<td>6.5 ± 1.2</td>
<td>5.6 ± 1.5</td>
<td>21.4 ± 3.4</td>
</tr>
<tr>
<td>Parahypoglossal nucleus</td>
<td>6.8 ± 1.3</td>
<td>3.3 ± 0.4</td>
<td>12.2 ± 2.1</td>
</tr>
<tr>
<td>Locus ceruleus</td>
<td>23.3 ± 2.7</td>
<td>13.5 ± 2.1</td>
<td>87.1 ± 19.6</td>
</tr>
<tr>
<td>Cerebellar cortex</td>
<td>2.8 ± 0.5</td>
<td>2.9 ± 0.3</td>
<td>8.4 ± 2.3</td>
</tr>
<tr>
<td>Spinal cord</td>
<td>3.7 ± 0.7</td>
<td>2.8 ± 0.2</td>
<td>1.2 ± 0.4</td>
</tr>
<tr>
<td>Anterior hypothalamus</td>
<td>13.6 ± 1.2</td>
<td>14.7 ± 1.8</td>
<td>19.0 ± 3.5</td>
</tr>
<tr>
<td>Posterior hypothalamus</td>
<td>9.7 ± 0.6</td>
<td>7.3 ± 0.6</td>
<td>6.6 ± 1.4</td>
</tr>
</tbody>
</table>

Values are means ± SEM and each is based on eight determinations.

supernatant by a modification of the method of Shiman et al. (1971) as fully described previously (Petty and Reid, 1979). This method used 4C-tyrosine as substrate and 6-MPH, (6-methyl-5,6,7,8-tetrahydropterine 2HCl) as co-factor, and the amount of 4C-DOPA formed was determined by liquid scintillation spectrometry after alumina extraction.

For PNMT assay, brainstem nuclei and cerebellar cortex were homogenized in 100 μl, and hypothalamus and spinal cord in 10 volumes of 0.2 M Tris-HCl buffer, pH 8.6, containing 0.2% Triton X-100. After the removal of 10 μl of homogenate for protein estimation, the samples were centrifuged as described above. PNMT activity was measured in 50 μl of supernatant by a modification of the method of Saavedra et al. (1974) as fully described previously (Petty and Reid, 1978).

In all experiments, eight sham-operated and eight denervated rats were killed on the same day and assayed in the same batch. Since the assays for chronic and acute groups were carried out in different batches and at different times, the values for the denervated rats are expressed as a percentage of the values obtained in the corresponding region of the appropriate (acute or chronic) sham-operated group; absolute values for the sham-operated rats are shown in Table 1. The significance of differences between denervated and control rats were calculated from the absolute data, using means ± SEM and Student's t-test for unpaired data.

Results

Blood Pressure and Heart Rate

Systolic pressure was measured in 15 sham-operated and 15 denervated rats 1 day before operation and at various intervals during the 28 days following operation, as shown in Figure 1. The systolic pressure was similar in the two groups preoperatively. Although there was little change in the sham-operated control rats after operation, the pressure was significantly increased in the denervated rats at all times tested during the month after the operation (Fig. 1). The increase in pressure was maintained unabated at the end of the 28-day period.

The heart rate was similar in the sham and denervated groups before operation and did not change significantly in the control rats during the 28 days following the sham operations. However the heart rate did rise significantly in the denervated rats. One week after operation, the heart rate was 453 ± 10 beats/min in the denervated rats compared with 385 ± 6 beats/min in the sham-operated controls; at 4 weeks, these values were 432 ± 6 and 364 ± 7 beats/min, respectively.

Norepinephrine Concentrations

One week after operation, the norepinephrine concentration was significantly greater in the de-
nervated rats compared to the sham-operated rats in the lateral reticular nucleus, the parahypoglossal nucleus, and the cerebellar cortex [158%, 167%, and 171% of control, respectively (Fig. 2)]. On the other hand, the norepinephrine concentration in denervated rats was significantly lower at this time in both the anterior and posterior hypothalamus [65% and 72% of control, respectively (Fig. 2)].

Four weeks after operation, these differences in norepinephrine concentration between denervated and control rats were no longer evident. The only significant difference at this time was in the spinal cord, where the norepinephrine concentration of the denervated, hypertensive rats was only 80% of the value in control rats ($P < 0.05$; Fig. 2).

**TH Activity**

In sham-operated rats, TH activity ranged from $1.4 \pm 0.4$ pmol $^{14}$C-DOPA/mg protein per hour in the spinal cord to $242.4 \pm 33.7$ nmol/mg protein per hour in the locus ceruleus (Table 1). One week after operation, TH was significantly greater in the denervated rats compared with the sham-operated rats in four regions: the nucleus of the solitary tract (188%), the parahypoglossal nucleus (254%), the locus ceruleus (191%), and the posterior hypothalamus (225%), as shown in Figure 3.

Four weeks after operation there were no significant differences between the TH activity of corresponding regions in the two groups of rats (Fig. 3).

**PNMT Activity**

PNMT activity in sham-operated control rats ranged from $4.7 \pm 0.8$ pmol phenylethanolamine/mg protein per hour in the cerebellar cortex to $63.7 \pm 3.7$ pmol/mg protein per hour in the anterior hypothalamus (Table 1). One week after operation, the only significant difference between the two groups of rats was in the posterior hypothalamus where PNMT activity was significantly lower in the denervated group compared to the shams (59% of control; Fig. 4).

Four weeks after operation, however, the PNMT activity was significantly different in three regions in the denervated rats compared with the control group. It was raised to 159% of control in the spinal cord of denervated rats and reduced to 69% and 64% of control in the anterior and posterior hypothalamus, respectively (Fig. 4).

**Discussion**

Systolic blood pressure and heart rate measured using the tail cuff method in conscious rats, were found to be persistently elevated at all times tested in the 28-day period after peripheral denervation of the carotid sinus and aortic baroreceptors (Fig. 1), as has been previously reported (Krieger, 1964;...
It is difficult to interpret changes in endogenous catecholamine concentration in terms of alterations in neuronal activity (Chalmers, 1975). Thus, in previous experiments in the rabbit, baroreceptor denervation did not produce any change in norepinephrine content in the spinal cord or hypothalamus, even though there were marked increases in norepinephrine turnover in these areas, as measured by the disappearance of tritiated norepinephrine (Chalmers and Wurtman, 1971). In the present paper there is a lack of correlation between changes in norepinephrine concentration and changes in TH activity. For example, in the acute experiments, there is a significant increase in norepinephrine concentration in the lateral reticular nucleus without a matching increase in TH activity, whereas the reverse is observed in the locus ceruleus (Figs. 2 and 3). It should be stressed that different parameters of neurotransmitter metabolism reflect different aspects of neural activity, and that the endogenous concentration of a neurotransmitter such as norepinephrine should not be used by itself as an index of the rate of nerve activity (Chalmers, 1975).

However, there is evidence that the activity of synthesizing enzymes such as tyrosine hydroxylase may reflect the rate of synthesis of transmitters and, thus, the frequency of neuronal discharge (Sedvall et al., 1968; Theirry et al., 1968; Chalmers, 1975). The interpretation of changes in PNMT activity is more difficult in that there are no published data on the correlation between the activity of this enzyme, the release of epinephrine, and the discharge rate of adrenergic neurones. However, it is generally assumed that the situation for central adrenergic neurones is analogous to that for noradrenergic neurones, and that the activity of the enzyme PNMT reflects the activity of adrenergic nerves (Saavedra et al., 1974, 1976).

Whereas changes in TH activity could reflect the activity of neurones releasing norepinephrine, epinephrine, or dopamine, it seems likely that in the present experiments they predominantly reflect changes in noradrenergic neurones. This is because the CNS regions examined are very much richer in nerve endings of neurones releasing norepinephrine than in nerves using epinephrine or dopamine (Versteeg et al., 1977). In addition, in some cases, as in the posterior hypothalamus at 7 days (Figs. 3 and 4), it is clear that the raised TH levels in the denervated rats reflect increased activity in noradrenergic neurones only, since the simultaneous reduction in PNMT activity indicates a reduction in the activity of adrenergic neurones.

The changes in norepinephrine concentration and TH activity in the brain stem and hypothalamus of denervated rats compared to controls were observed only 7 days after operation and had disappeared at 28 days. This suggests that central noradrenergic neurones in these areas may well participate in the initiation rather than in the maintenance of neurogenic hypertension. They could well form an element in those central connections of arterial baroreflexes responsible for adaptation and resetting, since these processes are known to occur over a similar time course (Korner, 1971). The one exception found in the present experiments was in the spinal cord, where the norepinephrine concentration was decreased in denervated rats at 28 days, suggesting a role for bulbospinal noradrenergic neurones in the long-term mechanisms responsible for neurogenic hypertension.

Central adrenergic neurones appear to play a more important role in the long-term response to baroreceptor denervation. Seven days after denervation, PNMT activity was different only to that found in sham-operated rats in the posterior hypothalamus, whereas at 28 days significant differences were found both in the anterior and posterior hypothalamus and in the spinal cord (Fig. 4). This time course is similar to that of changes in brain stem PNMT activity in the "one-kidney" rat with renovascular hypertension (Petty and Reid, 1978) and suggests that central adrenergic neurones may...
have a greater role in the maintenance than in the initiation of experimental hypertension. The fact that hypothalamic PNMT activity is decreased in this model, whereas it is increased in the "one-kidney" model (Petty and Reid, 1978), is entirely consistent with a baroreflex-dependent effect. In the neurogenic rat, arterial baroreceptor input has been eliminated by denervation, whereas in the one-kidney model of hypertension, baroreceptor input is accentuated. The fact that PNMT activity was increased in the spinal cord but not the lateral reticular nucleus of the chronically deafferented rats probably reflects the lack of coincidence of these two populations of neurones. Thus catecholaminergic neurones traveling down the cord do not all originate from the lateral reticular nucleus. Furthermore, the neuronal catecholamine in the lateral reticular nucleus is contained in both cell bodies and nerve endings, whereas all the neuronal catecholamine in the cord is contained in nerve endings.

These experiments confirm the importance of the hypothalamus in the arterial baroreceptor reflex and suggest that both adrenergic and noradrenergic nerves synapsing in the hypothalamus contribute to baroreflex function and to the increase in blood pressure seen after deafferentation of the baroreceptors. It is interesting that there was an increase in TPF activity accompanied by a simultaneous decrease in PNMT activity in the posterior hypothalamus of denervated rats at 7 days; this suggests that neurogenic hypertension may involve a reciprocal increase in the activity of noradrenergic hypothalamic neurones accompanied by a decrease in the activity of adrenergic hypothalamic nerves.

Finally, whereas PNMT activity in the denervated rats was decreased in the hypothalamus at 28 days, it was elevated in the spinal cord (Fig. 4). This is a timely reminder that although the activity of some central adrenergic nerves, such as those in the hypothalamus, may have a vasodepressor effect, in other areas such as the spinal cord, central adrenergic nerves may well act to elevate arterial pressure.

Acknowledgments

We are grateful to Beverly Fraser for her excellent technical assistance and to Professor C.T. Dollery for his support.

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Cardiac Atrial Myosin Adenosine Triphosphatase of Animals and Humans

Distinctive Enzymatic Properties Compared with Cardiac Ventricular Myosin

YOSHIO YAZAKI, SEIGO UEDA, RYOZO NAGAI, AND KAZUYUKI SHIMADA

SUMMARY  Cardiac myosin obtained from atria had a higher Ca\textsuperscript{2+}-activated ATPase activity than did cardiac myosin from ventricles in various species of animals and in humans. The increased specific activity of Ca\textsuperscript{2+}-activated adenosine triphosphatase (ATPase) of atrial myosin appeared to correlate with the level of the activity of ventricular myosin ATPase in the animal, since the same order in ATPase activity, as observed in ventricular myosins from various animals, was noted in atrial myosins. The enzymatic properties of atrial myosin also were characterized by no activation by N-ethylmaleimide, low activating energy, and a lower rate of inactivation at alkaline pH compared with the same properties of ventricular myosin. These findings suggest a difference in the myosin molecule at or near the active site, involving some sulfhydryl groups, between the two types of cardiac myosin. The Mg\textsuperscript{2+}-activated ATPase activity, both in the presence and absence of actin (which is thought to be closely related to the basic contraction mechanism), also was enhanced in atrial myosin. Thus, the ATPase activities of atrial and ventricular myosins were different with special reference to the reaction pathway involving calcium and magnesium ions and appear to account for the difference in the velocity of contraction between the atria and the ventricles.

WE HAVE demonstrated a difference in adenosine triphosphatase (ATPase) activities of cardiac myosins from different species of animals; cardiac myosin obtained from rats and mice (smaller animals) had a higher ATPase activity in the presence of calcium ions than did cardiac myosin from rabbits and dogs (larger animals) (Yazaki and Raben, 1974). Comparative study on enzymatic properties of these cardiac myosins suggested a difference in the myosin molecule at or near the active site involving the SH thiols. Also, we have shown that the pattern of enzymatic properties of cardiac myosin would be affected by the thyroid state (Yazaki and Raben, 1975). In view of the evidence that myosin ATPase activity is related closely to the intrinsic speed of muscle contraction (Barany, 1967), these differences in enzymatic activities may be reflected in the contractility of the corresponding cardiac muscles.

Recently, a high level of ATPase activity of myosin obtained from atrial muscle of dog and calf hearts has been reported by our laboratory (Yazaki et al., 1977) and by others (Long et al., 1977; Flink et al., 1978). The experiments presented in this paper attempt to characterize in greater detail enzymatic activities of atrial myosins from various animals and from humans and to determine the physiological significance of this observation with special reference to actin activation of myosin ATPase activity.

Methods

Myosin Isolation

Cardiac myosin was prepared from rat, rabbit, dog, and human hearts. Fresh heart muscle tissue was obtained from dogs, anesthetized with sodium...
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Circ Res. 1979;45:516-522
doi: 10.1161/01.RES.45.4.516

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4371

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