Evidence for the participation of sodium-sensitive pontine catecholaminergic neurons in the maintenance of DOCA-sodium hypertension in rats

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Summary
1. A reduced noradrenaline turnover rate has been previously demonstrated in the brain stem of deoxycorticosterone acetate (DOCA)-sodium hypertensive rats. In the present study, the turnover rate was measured in smaller brain regions and the effect of sodium depletion was studied on the turnover rate of these regions.
2. Catecholamine turnover rate was significantly reduced only in the pons, was slightly but not significantly reduced in the thoracic spinal cord and was normal in the upper and lower medulla oblongata, in the mesencephalon, in the hypothalamus and in the telencephalon.
3. The administration of a sodium-free diet for 3 weeks lowered significantly the blood pressure and concomitantly accelerated the turnover rate in the pons area of DOCA hypertensive rats.
4. It is concluded that pontine catecholaminergic fibres, sensitive to sodium balance, might be involved in the maintenance of DOCA-sodium hypertension in the rat.

Key words: catecholamine turnover, central nervous system, experimental hypertension, sodium depletion, sympathetic nervous system.

Introduction
The activation of the cardiovascular sympathetic nervous system and adrenal medulla has been well documented during the development of deoxycorticosterone acetate (DOCA)-sodium hypertension (de Champlain & Van Ameringen, 1975). Moreover, the state of sodium balance was found to be closely related to the peripheral sympathetic tone (de Champlain, Krakoff & Axelrod, 1969). The changes in peripheral sympathetic activity in DOCA-sodium hypertension were suspected to be under the influence of the central nervous system since ganglionic blockade (de Champlain, Krakoff & Axelrod, 1968) or spinal cord section at the level of C6-C7 (Van Ameringen, de Champlain & Imbeault, 1977) resulted in the normalization of blood pressure and noradrenaline turnover rates in peripheral organs of DOCA-sodium hypertensive rats.

The participation of brain catecholaminergic fibres in the regulation of the cardiovascular sympathetic system has been well demonstrated (Fuxe, Bolme, Jonsson, Agnati, Goldstein & Hökfelt, 1979). A decrease in the noradrenaline turnover rate was observed in the brain stem (Nakamura, Gerold & Thoenen, 1971; Yamori, Ooshima & Okamoto, 1973; Van Ameringen et al., 1977) and in the hypothalamus (Nakamura et al., 1971) of DOCA-sodium hypertensive rats. These changes did not appear to be secondary to the elevation of blood pressure since the reduced turnover rate in the brain stem of DOCA-sodium hypertensive rats persisted after important reduction of the blood pressure after administration of a ganglion blocker (Nakamura et al., 1971) or after spinal cord section (Van Ameringen et al., 1977). More recently, increased activity of the adrenaline-forming enzyme, phenylethanolamine-N-methyltransferase (PNMT) and increased adrenaline levels were observed in the areas A1 and A2, the locus coeruleus and the nucleus commissuralis during the development of DOCA-sodium hypertension (Saavedra, 1979). These observations are compatible with the hypothesis that a change in activity in certain
catecholaminergic pathways in the brain stem of DOCA–sodium hypertensive rats may be the primary dysfunction leading to the development of hypertension.

In the present study, the catecholamine turnover rate was studied in smaller brain-stem areas to attempt a better localization of the site of dysfunction. Moreover the effect of sodium depletion was studied on the turnover rate to determine whether the fibres demonstrating dysfunction were sensitive to sodium balance and whether this dysfunction was reversible.

**Methods**

Male Sprague–Dawley rats weighing 80–90 g had their right kidney removed. A group of these animals were made hypertensive by weekly subcutaneous injection of 10 mg of a suspension of deoxycorticosterone pivalate (Ciba) and 1% sodium chloride solution to drink ad libitum for 7 weeks. In the sodium-depletion experiment, rats given DOCA and sodium chloride for 4 weeks and normotensive rats were given one intraperitoneal injection (10 mg/kg) of the diuretic agent, ethacryninc acid (Edecrin, Merck, Sharp & Dohme). The DOCA was stopped in hypertensive animals and the animals were fed on a synthetic sodium-free diet (Nutritional Biochemical Co.) for the following 3 weeks. The control animals for these studies were fed for 3 weeks on the same synthetic diet to which 22 g of NaCl was added per kg of diet food.

The systolic blood pressure and heart rate were measured in unanaesthetized pre-warmed animal by mean of a pulse transducer applied to the tail and recorded on a Grass model 7B polygraph.

Noradrenaline turnover rates were studied after inhibition of the noradrenaline synthesis by intraperitoneal injection of DL-α-methyl-p-tyrosine methyl ester/HCl (Labkemia AB, Sweden). The drug was diluted in sodium chloride solution (154 mmol/l) and injected at the dose of 300 mg/kg every 3 h. The animals were killed by decapitation at 0, 3 and 6 h after the first injection. A section of the duodenum, the thoracic spinal cord (T1–T12) and the brain without the cerebellum were quickly removed and chilled on crushed ice. The brain was dissected on an ice-cold plate according to the following anatomical guidelines. The medulla oblongata was sampled between the first cervical nerve and the ponto-bulbar sulcus. The medulla oblongata was separated into a lower and upper part by a section at the level of the obex. The pons was sampled between the ponto-bulbar sulcus and the rostral limits of the transverse pontine fibres. The mesencephalon was sampled from the transverse pontine fibres to the rostral limits of the colliculi. The hypothalamus was sampled between the optic chiasma and the thalamus. The telencephalon was constituted by the remaining brain tissue less the cerebellum.

The endogenous catecholamine levels were determined by the radioenzymatic technique of Coyle & Henry (1973). The turnover of catecholamine was determined by calculating the decline of endogenous catecholamine levels expressed as a percentage of the initial value. Curves were derived from the weighed least-square approximation of the log of the data point and the half-life values were calculated from these curves. The significance between the half-life curves was calculated by using the regression analysis test.

**Results**

The average systolic blood pressure in rats given DOCA–sodium for 7 weeks was 212 ± 0.8 mmHg compared with 120 ± 0.8 mmHg for age-matched control rats. At that time, the catecholamine turnover was significantly decreased in the pons (half-life: 5.0 vs 3.2 h, P < 0.05) and slightly decreased in the thoracic spinal cord (6.4 vs 4.5 h) of DOCA–sodium rats. However, the turnover rates did not differ from control rats in the upper and lower medulla oblongata, in the mesencephalon, in the hypothalamus and in the telencephalon of hypertensive rats. Initial endogenous levels were only increased in the mesencephalon but were significantly increased in the pons and thoracic spinal cord of DOCA–sodium rats 6 h after administration of α-methyl-p-tyrosine.

When normotensive and DOCA–sodium rats were subjected to a sodium-free diet for 3 weeks, the systolic blood pressure was lower by 52 mmHg in hypertensive rats and by 11 mmHg in normotensive rats. Concomitantly the half-life of catecholamines in the pons fell from 6.1 to 3.4 h in the hypertensive animals, thus restoring the turnover rate to normal. In normotensive rats similarly treated, the half-life slightly increased from 3.8 to 5.5 h in the pons (Fig. 1). Endogenous catecholamine levels were greater by 29% in the pons of hypertensive rats on a sodium-free diet. In the periphery, the turnover rate, which was increased in the intestine of DOCA–sodium animals, was restored to normal when these animals were given a sodium-free diet.

**Discussion**

This study confirms the existence of a reduced turnover rate in the brain-stem area of DOCA–
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100
80
60
40
20
0

*P < 0.05

% of mean initial value

0 3 6

Hours

Sodium depletion and brain catecholamines

FIG. 1. Catecholamine turnover rates in the pons of normotensive rats (●) and DOCA–sodium hypertensive rats (○) given a synthetic sodium-free diet or the same diet to which sodium (22 mg/kg) was added (control diet). Each point plotted represents the mean ± se as percentage of initial values. On the control diet the mean systolic blood pressure and heart were 204 ± 4 mmHg and 459 ± 10 beats/min for 15 DOCA–sodium hypertensive rats and 135 ± 3 mmHg and 485 ± 6·0 beats/min for 15 normotensive rats. On the sodium-free diet, blood pressure and heart rate were 152 ± 1 mmHg and 474 beats/min in 18 DOCA hypertensive rats and 124 ± 2 mmHg and 478 ± 9·0 beats/min in 14 normotensive rats. Initial endogenous catecholamine levels were 673 ± 80 ng/g in DOCA–sodium hypertensive rats and 791 ± 29 ng/g in normotensive rats on the control diet. On the sodium-free diet, initial levels were 869 ± 79 ng/g for the hypertensive rats and 690 ± 109 ng/g for the normotensive rats.

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sodium hypertensive rats but it appears that this abnormality is restricted to the pontine area and probably to the thoracic spinal cord as well. Since it was previously demonstrated that the decreased turnover rates persisted in the brain stem of DOCA–sodium rats after ganglion blockade (Nakamura et al., 1971) or after spinal cord section (Van Ameringen et al., 1977), it is unlikely that these changes are secondary to the elevation of blood pressure. A similar decrease in noradrenaline turnover was also observed in the brain stem of spontaneously hypertensive rats (Yamori et al., 1973). Since an increased peripheral sympathetic activity has been demonstrated in spontaneously hypertensive rats and DOCA–saline hypertension, it is tempting to postulate a causal relationship between the reduced catecholamine turnover in the brain stem and the increased turnover observed in peripheral vascular organs in these two experimental models. These findings are compatible with the existence of a central noradrenergic vasodepressor system postulated to be localized in the area of vasomotor centres of the medulla oblongata (Chalmers, 1975). Our observation of normal turnover rates in the upper and lower medulla oblongata seems to exclude this possibility. However, a dysfunction in adrenaline fibres of the medulla oblongata cannot be excluded by the present study since the technique of Coyle & Henry (1973) used for the measurement of catecholamine levels does not differentiate between adrenaline and noradrenaline. Moreover the direct monosynaptic connections between the nucleus tractus solitarii and the locus coeruleus localized in the pons or the intermediolateral nucleus of the spinal cord (Palkovits, Mezey & Zaborsky, 1979) support the possibility that a primary dysfunction affecting the nucleus tractus solitarii in the medulla oblongata could be reflected by changes in turnover rates at the level of the pons and spinal cord. Conversely, the possibility that a primary dysfunction in the locus coeruleus or related area of the pons could alter the function of the nucleus tractus solitarius or other vasomotor areas cannot be excluded by the present study.

It was previously demonstrated that sodium depletion could normalize blood pressure and the noradrenaline turnover rates in peripheral vascular organs of DOCA-hypertensive rats (de Champlain et al., 1968, 1969). The present study suggests that these peripheral changes could be secondary to an effect of sodium balance on central catecholamine fibres of the pontomedullary vasomotor centres since a marked activation of the turnover in pontine catecholaminergic fibres of DOCA-hypertensive rats was associated with a lowering of blood pressure and a decrease in peripheral sympathetic activity during sodium depletion. The causal relationship between these changes is hard to demonstrate...
but, since brain-stem catecholamine turnover appeared to be unaffected by changes in peripheral blood pressure (Van Ameringen et al., 1977), it is unlikely that the present finding in the brain stem would be a consequence of the lowering of blood pressure by sodium depletion. It is also of interest that sodium depletion resulted in a 29% increase in the endogenous content of the pons in the hypertensive animal. These findings are consistent with the observations of an inverse correlation between brain-stem norepinephrine levels and blood pressure in spontaneously hypertensive rats treated with L-dopa and monoamine oxidase inhibitors (Yamori, de Jong, Yamabe, Lovenberg & Sjoerdsma, 1972). The mechanism whereby sodium depletion would change the catecholamine content and turnover rate in the pons remains totally obscure at this point but deserves further investigation.

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References


