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The effects of enalapril maleate and cold stress exposure on tyrosine hydroxylase activity in some rat tissues

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Enalapril is a highly specific and competitive inhibitor of angiotensin-I converting enzyme (ACE) and thus belongs to the category of ACE inhibitors. The beneficial effects of ACE inhibitors appear to result primarily from the suppression of the plasma renin–angiotensin–aldesterone system. This study was designed to detect the effects of enalapril maleate and cold stress on tyrosine hydroxylase (TH) activity in adrenal medulla, heart and hypothalamus in rat. In cold stress treatment (exposed to 8°C cold for 48 h) TH activity was found to be raised significantly ($p < 0.05$) in adrenal medulla, hypothalamus and heart tissues. In the adrenal medulla, hypothalamus and heart tissues, TH activity of enalapril maleate treated rats (10 mg kg^{-1} body weight) group was not raised significantly ($p > 0.05$). Following intraperitoneal injection of enalapril maleate (10 mg kg^{-1} body weight) the rats were exposed to 8°C cold for 48 h. After cold stress and enalapril maleate treatment no statistically significant change in tyrosine hydroxylase activity was detected in adrenal medulla, hypothalamus or heart ($p > 0.05$). The results of our studies show that enalapril maleate blocks the effect of cold stress on the regulation of TH activity. Copyright © 2005 John Wiley & Sons, Ltd.

KEY WORDS — enalapril maleate; cold stress; tyrosine hydroxylase; rat

ABBREVIATIONS — ACE, angiotensin converting enzyme; TH, tyrosine hydroxylase; NE, norepinephrine; EPI, epinephrine; CA, catecholamine

INTRODUCTION

High blood pressure can damage the blood vessels of the brain, heart, and kidneys, resulting in a stroke, heart failure, or kidney failure. By lowering blood pressure, enalapril maleate, a diuretic, can help reduce the risk of damage to such organs and tissues. Experimental studies have shown that angiotensin-converting-enzyme inhibitors have positive effects on the circulation. Enalapril maleate, which is an angiotensin converting enzyme inhibitor, is used as an antihypertensive drug and blocks angiotensin-I conversion to angiotensin-II[1–3] by its action on angiotensin converting enzyme (ACE).[1–3] Angiotensin-II elevates the release of norepinephrine from terminal adrenergic neurons.[3,4] Moreover, angiotensin-II elevates the release of epinephrine and norepinephrine from adrenal medulla and stimulates the autonomic ganglions in the peripheral autonomic nerve system.[1,4,5] Tyrosine hydroxylase (TH) is an enzyme which plays a central role in neurotransmission and hormonal function of catecholamines.[6] TH is the rate-limiting enzyme in the synthesis of the catecholamines dopamine, epinephrine and norepinephrine. Therefore the regulation of TH represents the central means for controlling the synthesis of these important catecholamines. TH has a large molecular diversity, resulting from differential splicing of its mRNA, which is tissue specific and can result in long-term changes in the activity of the enzyme. In addition, it affects the availability of neurotransmitter substances at various synapses. Dihydroxyphenylalanine (DOPA), dopamine, norepinephrine (NE) and epinephrine (EPI) are synthesized in the catecholamine pathway.[7] Chromaffin cells in the adrenal medulla release catecholamines. Both synthesis and

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release of catecholamines rise in these cells as a result of the increase in TH activity and TH mRNA.[8]

Physical activity and physiological stress lead to stimulation of the adrenergic system. The catecholamines which play an adrenergic role are synthesized in brain, adrenal medulla and heart.[9] Cold exposure causes the release and synthesis of catecholamines in all these tissues. Furthermore, in cold exposure, the level of TH mRNA increases and so the TH activity increases.[10–14]

No published studies have described the effect of enalapril maleate on TH activity after cold exposure and none of them has demonstrated an accumulation in the HPA (hypothalamic–pituitary–adrenal) axis. In the present study, TH activity has been assessed in the adrenal medulla, hypothalamus and heart of the rat by a radiochemical method. The aim of the present study was to evaluate the effects of cold stress, enalapril maleate and cold stress + enalapril maleate on TH activity in the adrenal medulla, hypothalamus and heart and to compare the effects of cold-exposed and enalapril maleate-treated animals.

MATERIALS AND METHODS

Setting of groups

Three-month-old Fischer-344 rats (body weight 200–250 g) were used in these experiments. Water and food were provided *ad libitum* and they lived in a 12 h light–dark cycle at room temperature during the study. In the study, 32 healthy Fischer-344 rats were divided into four groups, each consisting of eight animals. Animals in group I (control group) were used as a control; animals in group II (cold stress group) were exposed to 8°C for 48 h; animals in group III (enalapril maleate group) were injected (IP) with enalapril maleate (10 mg kg^{-1}); rats in group IV (cold stress + enalapril maleate), were also injected (i.p.) with the drug at 10 mg kg^{-1}. After enalapril maleate injection, the rats were exposed to 8°C for 48 h.

Sample preparation and methods

After the treatments, rats were anaesthetized with 75 mg kg^{-1} sodium pentobarbital, their chests were opened the vena cava was cut and 30 ml of 0.9% NaCl was injected into the heart to rinse blood from the body. The adrenal medulla, hypothalamus and heart were removed and frozen in liquid nitrogen. Tissues were stored at −40°C until used. Tissues were weighed then homogenized in 100 µl of 2 mM phosphate buffer, pH 7.4. Homogenates were used for determination of total protein[15] and measurement of TH activity. TH activity was determined based on a modification of the assay as described previously.[16] Briefly 25-µl homogenates were analysed in the presence of 6-MPH_4 (1.5 mM) and [3,5 ^{3}H] tyrosine (100 µM: 1 µCi per reaction), in a total volume of 50 µl for 15 min at 37°C. Unbound ^3H_2O was analysed by scintillation spectrometry (Beckman LS 5000 TD).

Statistical analyses

The data were evaluated and any differences between the groups were tested for statistical significance using ANOVA and LSD. The results are shown as means ± SE. The significance level was defined as $p < 0.05$.

RESULTS

In determining the effects of cold stress and enalapril maleate on catecholamine (CA) biosynthesis TH activity was measured in adrenal medulla, hypothalamus and heart tissues after 48 h cold exposure with/without enalapril maleate. The effects of enalapril maleate and cold stress on TH activity are shown in Table 1. Cold exposure produced significant effects on TH activity in various tissues of the rat. The analysis also showed a highly significant effect of enalapril maleate + cold stress on TH activity. Multiple comparisons by an LSD test showed that between the control and enalapril maleate + cold stress group, TH activity did not vary significantly within 48 h but TH decreased significantly in enalapril maleate-treated animals in comparison with cold treated animals ($p<0{\cdot}05$; Table 1).

TH activity of the adrenal medulla of Fischer-344 rats increased significantly from 18.5 ± 0.6 to 39.4 ± 2.7 nmol mgP^{-1} h^{-1}, after cold stress treatment

Table 1. TH enzyme activities in adrenal medulla, hypothalamus and heart of Fischer-344 rats which were treated with cold stress and enalapril maleate (mean ± SE)

	TH enzyme activity (nmol mgP^{-1} h^{-1})		
	Adrenal medulla	Hypothalamus	Heart
Control	18.5 ± 0.6	18.5 ± 0.8	13.6 ± 0.7
Cold stress	39.4 ± 2.7	37.8 ± 2.3	23.4 ± 2.2
Enalapril maleate	20.1 ± 1.7	22.3 ± 0.5	15.2 ± 1.4
Cold stress + enalapril maleate	17.5 ± 0.3	18.2 ± 1.3	13.6 ± 1.1

($p < 0.05$; Table 1). In the adrenal medulla, TH activity of the enalapril maleate-treated group was 20.1 ± 1.7 nmol $mgP^{-1}\,h^{-1}$. This difference was not significant ($p > 00.05$; Table 1). TH activity of the enalapril maleate + cold stress treated group was 17.5 ± 0.3 nmol $mgP^{-1}\,h^{-1}$. Compared with the control group, this decline was not significant ($p > 00.05$; Table 1).

In the hypothalamus, TH activity increased from 18.5 ± 0.8 to 37.8 ± 2.3 nmol $mgP^{-1}\,h^{-1}$ after cold stress treatment group (significant at $p < 0.05$; Table 1). In the enalapril maleate treatment group, TH activity was 22.3 ± 0.5 nmol $mgP^{-1}\,h^{-1}$ i.e. not significantly different ($p > 0.05$; Table 1). TH activity of enalapril maleate + cold stress group was 18.2 ± 1.3 nmol $mgP^{-1}\,h^{-1}$. There was no significant change between the control and the treatment groups ($p > 0.05$; Table 1).

In the heart, TH activity increased from 13.6 ± 0.7 to 23.4 ± 2.2 nmol $mgP^{-1}\,h^{-1}$ (significant at $p < 0.05$). TH activity of the enalapril maleate treatment group was 15.2 ± 1.4 nmol $mgP^{-1}\,h^{-1}$ i.e. not significantly different from the control value ($p > 0.05$; Table 1). In the cold stress + enalapril maleate group TH activity was 13.6 ± 1.0 nmol $mgP^{-1}\,h^{-1}$ (Table 1). This was not statistically significant ($p > 0.05$; Table 1).

DISCUSSION

In cells that synthesize and secrete dopamine, norepinephrine, and epinephrine, the neurotransmitter levels are precisely controlled through the regulation of tyrosine hydroxylase, the rate-limiting enzyme in this neurotransmitter biosynthetic pathway. It has been reported that TH mRNA and TH activity increase in the adrenal glands of rats that are exposed to cold.[10,12] During chronic cold exposure of rats, release of adrenal catecholamines occurs. Increase in adrenal TH activity supports this increase in catecholamines release during cold exposure.[8,10–14]

Enalapril maleate which is an angiotensin converting enzyme inhibitor reduces blood pressure as it decreases peripheral vein resistance. After cold exposure, TH enzyme activity in adrenal medulla, hypothalamus and heart of rats increased. It can be hypothesized that if TH activity increases, catecholamine production would also increase. On the other hand, angiotensin converting enzyme inhibitors reduce norepinephrine, epinephrine, angiotensin II, aldesterone and renin levels of these increased catecholamines. Enalapril maleate, which is an antihypertensive, has a blocking effect on angiotensin converting enzyme in the renin–angiotensin system. This renin–angiotensin system is connected with gene expression of biosynthetic enzymes of catecholamines.[17] Because angiotensin II has a blocking effect, it can also be said that enalapril maleate reduces vein pressure and blocks increases in TH activity.

Tyrosine-derived catecholamines, including DOPA, dopamine and NE synthesized within the hypothalamus and adrenal medulla are important stress hormones. In rats there is evidence that TH activity rises with cold stress but declines after treatment with enalapril maleate. Stress-related differences in TH activity of hypothalamus, adrenal medulla and heart are similar to those described in the published literature. The potential of a regulatory and compensating effect of enalapril maleate on blood pressure via a decrease of TH activity in adrenal medulla, hypothalamus and heart is reported for the first time in this study.

TH activity in the adrenal glands increased in the cold-exposed groups but enalapril maleate prevented the cold-induced increase. The protective effect of enalapril maleate in cold-induced hypertension may be related to a reduction in plasma renin activity and to a reduced responsiveness to angiotensin II.

In conclusion, cold stress increases TH activity and biosynthesis while it affects the adrenergic nervous system. So it can be said that cold stress elevates biosynthesis of catecholamines and the increased TH enzyme activity is blocked by enalapril maleate. Further studies and evaluation of different parameters are needed to identify the mechanism of adaptation of cold stress tolerance in mammals and to understand the exact role of the enalapril maleate in response to cold and aging. To our knowledge, this is the first report showing that enalapril maleate can result in decreased TH activity in heart, adrenal medulla and hypothalamus of cold-exposed rats.

The positive effect of enalapril maleate in cold-exposed animals may be related both to a reduction in TH activity and to a reduced responsiveness to angiotensin II. The results of our experiment suggest that enalapril maleate may play an important role in maintaining homeostasis as an antagonist substance.

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