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SHORT COMMUNICATIONS

Enzyme activity of an amine oxidase resistant to pargyline in the cardiovascular system of hypertensive rats

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Different amine oxidases have been defined by their specificity for substrates and for their sensitivity to inhibitor drugs. Recently, reports from several laboratories [1–4] have extensively described the features of a new kind of amine oxidase activity of cardiovascular tissue that deaminates phenylethylamine (PEA) and benzylamine (BA) and is resistant to acetylenic monoamine oxidase [monoamine: O_2 oxidoreductase (deaminating); EC 1.4.3.4; MAO] inhibitor drugs. Such an enzyme, which could be termed pargyline-resistant amine oxidase (PRAO) as it became evident to us for the first time in the presence of increasing concentrations of pargyline using heart tissue as enzyme source [5], has also been called clorgyline-resistant amine oxidase [2] and benzylamine oxidase [4] by other authors. PRAO exists in very low levels in the brain and at variance with the PEA- and BA-deaminating activity (type B MAO [6]) of this tissue, can be blocked by carbonyl and copper-chelating agents [3]. This enzyme activity, which has been reported to be widely distributed in rat and human tissues [4], may thus be considered a pyridoxal and copper-dependent amine oxidase.

The functional role of PRAO is still an open question. By studying a given enzyme activity in a pathological state, the functional significance of the catalytic protein can be approached. It is generally assumed that high blood pressure is, among other causes, due to changes in connective tissue metabolism. On the other hand, PRAO shows a relatively high concentration in bone, skin and muscle; that distribution might imply a role for the enzyme in connective tissue metabolism [7]. Interestingly, hydralazine, which is used for the treatment of some forms of hypertension [8], has been found to readily inhibit PRAO in rat aorta [9]. On these bases, PRAO activity was examined in two different experimental models of hypertension: the spontaneously hypertensive rat (SHR [10]) and in uniphrectomized rats made hypertensive by treatment with deoxycorticosterone acetate (DOCA) – salt [11].

Materials and methods

SHR and normotensive Wistar–Kyoto rats (WKR) were genetically related Wistar–Kyoto male rats [10]. Deoxycorticosterone acetate (DOCA; Sigma Chemical Co., Dorset, U.K.) salt hypertension [11] was produced in uniphrectomized 80–90 g male Wistar rats by twice-weekly subcutaneous injections of DOCA (5 mg/kg).

Arteries were homogenized with 20 volumes and hearts with 4 volumes of 67 mM phosphate buffer, pH 7.2. The supernatant fraction from centrifugation at 750 *g* for 10 min was used as the enzyme source. Homogenates were incubated at 37° for either 60 min with radioactive 0.2 m PEA ([1-^{14}C]-2-phenylethylamine; Radiochemical Centre, Amersham, U.K.), or 30 min with radioactive 0.05 M BA ([7-^{14}C]-benzylamine; Radiochemical Centre, Amersham, U.K.). Incubation mixtures always included 1 mM pargyline (generously supplied by Abbot Laboratories, North Chicago, IL). The reaction was stoped by adding 50 μl 60% $HClO_4$ and, according to the method published by Lewinsohn *et al.* [4], the radioactive deaminated reaction products were extracted into toluene (3 ml) and measured in a Packard Tri-Carb 2425 liquid scintillation counter. The enzyme assay was linear with protein and time of incubation. Protein was determined by the method of Lowry *et al.* [12].

The systolic arterial pressure of unanaesthetized rats was evaluated by an indirect method using a W & W blood pressure recorder [13]. A mean value from at least eight consecutive readings for each animal was used for computations.

Statistical data analysis was done by Student's *t*-test.

Results

Compared to normotensive animals (WKR), PRAO activity was shown to be enhanced by about 60% in the homogenates of the SHR mesenteric artery in animals with established hypertension when either PEA or BA was used as substrate (Fig. 1). In contrast, no difference between hypertensive and normotensive rats was detected when PRAO activity levels in the heart were compared using PEA as a substrate. The fraction of PEA-deaminating activity sensitive to pargyline (type B MAO), when expressed as nmole of product per mg protein per hr, did not differ in the mesenteric artery (SHR: 9 ± 2; WKR: 8 ± 1) or in the heart (SHR: 8 ± 1; WKR: 7 ± 1).

The blood pressure of uniphrectomized rats treated with DOCA–salt for 5–7 weeks increased significantly (185 ± 6 mm Hg) above the readings of normotensive animals (130 ± 6 mm Hg). In contrast to the genetically hypertensive rats, no difference was found when PRAO enzyme activity in DOCA–salt treated rats was compared to PRAO levels in normotensive rats used as controls (Fig. 1).

To determine whether the increase found in PRAO of SHR mesenteric artery was related to a higher affinity of this enzyme for its substrate or to a change in the V_{max}, a kinetic analysis in SHR and WKR mesenteric arteries was carried out. PEA was selected for this type of experiment since it is widely distributed in rat body tissues [14] while no endogenous levels of BA have been detected so far. By using different concentrations of PEA, Lineweaver–Burk plots indicated that in every case the V_{max} of the enzyme in SHR mesenteric artery homogenates was always higher than the value measured for the enzyme V_{max} in normotensive WKR rats (results no shown). No significant difference was found in the apparent Michaelis constants (K_m of approximately 65 μM) for PEA-deaminating activity in the two types of rats.

To see whether PRAO activity was somehow related to the hypertension development in spontaneous hypertension, SHR and WKR rats were killed at different ages and their PEA-deaminating activities assayed in the presence of 1 mM pargyline in homogenates of mesenteric artery (Fig. 2). At 1 month of age, SHR blood pressure did not show any significant difference compared to the readings obtained in normotensive WKR rats. Neither was any difference in PRAO activity found in the two groups of animals. Interestingly, when SHR rats had become hypertensive after 1 month, their mesenteric artery PRAO activity was significantly higher than that in age-matched WKR normotensive rats. In our SHR colony, arterial pressure enhanced until the age of 4–5 months and then stabil-

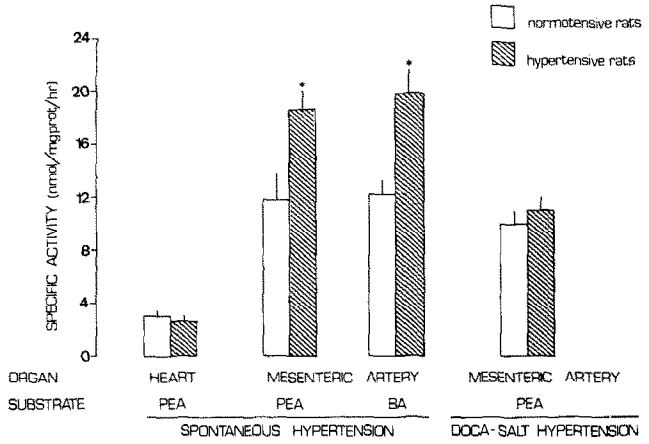

Fig. 1. Pargyline-resistant amine oxidase (PRAO) activity in heart and mesenteric artery of normotensive and hypertensive rats. Male spontaneous hypertensive rats (SHR) and normotensive Wistar–Kyoto rats (WKR), 7 months of age, were used. In the case of the DOCA–salt model of hypertension, male Wistar rats (80–90 g) were made hypertensive by subcutaneous injections of a suspension of DOCA, and by drinking a 1% NaCl solution for periods of 5–7 weeks. Enzyme activity was evaluated in the presence of 1 mM pargyline by using either 0.2 mM phenylethylamine (PEA) or 0.05 mM benzylamine (BA) as substrates. Values correspond to mean ± S.E.M. for groups of 8–10 animals. * $P < 0.05$ when compared with age-matched normotensive rats.

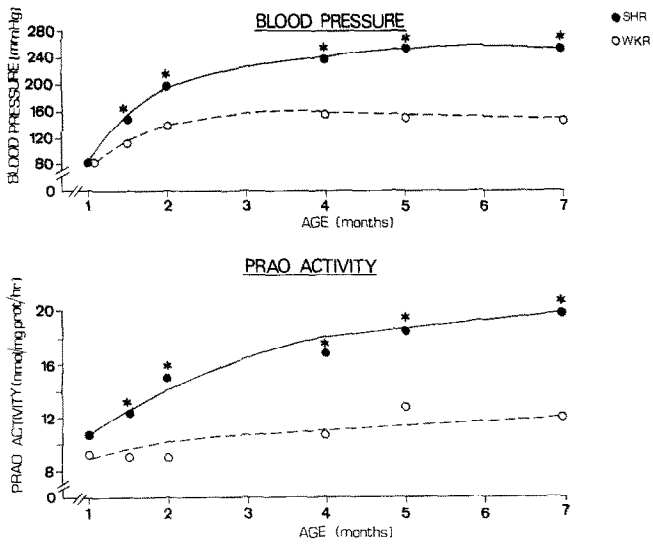

Fig. 2. Time course of arterial pressure and mesenteric artery PRAO in normotensive (WKR) and hypertensive (SHR) rats. Systolic blood pressure and mesenteric artery PEA-deaminating activity were evaluated at various times between 3 weeks and 7 months of age. Data are presented as the mean for groups of 5–8 animals. * $P < 0.05$ when compared with control animals.

ized for life at about 240 mm Hg. In parallel, SHR PRAO activity continued to increase until the rats were 4 months old and then remained stable, but was always significantly higher than in WKR rats up to the last measurement performed when rats were 7 months old.

Discussion

In SHR rats, PRAO activity increased steadily with age in homogenates of mesenteric artery but not in the heart (Figs. 1 and 2). In this regard, it is interesting that while cardiac output is normal in genetically hypertensive rats [15], increases in arterial pressure are linked to elevation in peripheral resistance. The constant increase of SHR blood pressure until the age of 4–5 months was always paralleled by an enhancement in mesenteric artery PRAO activity that remarkably did not show up until the rats became hypertensive (Fig. 2). Although other interpretations are possible, the kinetic analysis of the elevation in PRAO activity supports the hypothesis that the synthesis of enzyme protein may be stimulated in the hypertensive stage of SHR rats. Our results and reports by others suggest that by its distribution [7] and inhibitors [3] PRAO may be related to a connective tissue amine oxidase [16], presumably involved in connective tissue metabolism. The recent discovery [17] of clorgyline-resistant amine oxidase activity in rat brown adipose could be an extension to its existence in connective tissue since cells of adipose tissue are seen as modified fibroblasts [18]. Although at present the similarities of PRAO with either connective tissue amine oxidase, or lysyl oxidase or plasma amine oxidase

remain to be completely substantiated, it can be assumed that the pargyline-resistant enzyme activity may play a role in the cross-linking of collagen and elastin in blood vessels [3, 4, 17].

In DOCA–salt hypertensive rats, no alteration in mesenteric artery (Fig. 1) PRAO levels was detected. However, when the enzyme assay was performed when these rats were younger than SHR rats showing equivalent blood pressure readings, the difference in arterial pressure between normotensive and DOCA–salt hypertensive rats was highly significant. It should be taken into consideration that when enzyme activity was evaluated in these animals the circulating levels of the mineralocorticoid could still be high. In this regard, increased MAO activity in the rat heart has been observed after adrenalectomy [19]. On the other hand, in a study on the neuronal factors that participate in the development of hypertension, alterations of some of the enzymes involved in brain amine biosynthesis of SHR were reported [20] while, in turn, no parallel changes could be detected in DOCA–salt hypertensive rats.

In summary, compared to normotensive rats, PRAO activity was found to be enhanced in homogenates of mesenteric artery in genetically hypertensive rats. This increase in enzyme levels was correlated with the development of hypertension in this model. The reduction in blood pressure detected after administration of hydralazine, an inhibitor of cardiovascular PRAO [9], also supports the notion for a role of this enzyme in the SHR model of hypertension. The purification and further characterization of PRAO as well as the study of its distribution in different cell types of cardiovascular tissue may give an insight into the ethiology and treatment of some cardiovascular alterations.

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A study of amine oxidases in bleomycin-induced pulmonary fibrosis

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Bleomycin causes pulmonary injury and fibrosis in animals and man [1–5]. In rats, Counts *et al.* [6] showed bleomycin to increase lung lysyl oxidase activity. This amine oxidase promotes the cross-linking of collagen and elastin [7, 8]. Whether other lung amine oxidases are also affected has not been studied and forms the basis of the present investigation. In this regard, benzylamine oxidase (BzAO) is of particular interest since some workers have postulated an association between BzAO and connective tissue disorders [9, 10].

BzAO is distinct from lysyl oxidase [11] and is found in the serum, connective and cellular tissues of animals and man [8–10, 12–14]. Clinical studies show increased serum BzAO activity in patients with diagnosed fibrosis [9, 15, 16] including pulmonary fibrosis [17]. Conversely, serum BzAO is reported to be unchanged in chronic obstructive lung disease while decreased activity has been found in severely burnt patients and in cancer patients [17]. Thus, the association between serum BzAO activity and connective tissue proliferation remains tenuous and rests entirely upon assays of the serum enzyme derived from patient populations. It seemed to us that a more definitive test for a role of BzAO in connective tissue disorders could be obtained by assaying both serum and cellular BzAO in bleomycin-induced pulmonary fibrosis in rats. Monoamine oxidases (MAO) types A and B were also measured since a variety of amines have been linked to the pulmonary toxicity of bleomycin [18].

Materials and methods

Male Fischer rats (weighing 169 ± 9 g) were obtained from the Charles River Breeding Laboratories, Kingston, NY, and were barrier-maintained as described previously [4]. All animals were observed for 7 days prior to use, and bleomycin sulfate (640 μg/165 g body wt in 0.5 ml of sterile saline) or saline was quickly instilled intratracheally as described by Counts *et al.* [6].

Preparation of tissues. The animals were anesthetized