Table 2. Kinetic investigation of the release of oxygen from haemoglobin

	(A) Haemoglobin (2–8 μM)	(B) Haemoglobin plus spectrin	(B)/(A)	Haemoglobin plus lysozyme
pH = 6.2 (unbuffered)	47.9 sec^{-1}	37.5 sec^{-1}	0.78	–
pH 7.15 (0.1 M phosphate)	34.3 sec^{-1}	27.2 sec^{-1}	0.79	34.7 sec^{-1}
pH 7.3 (0.1 M Tris/HCl)	31.5 sec^{-1}	27.7 sec^{-1}	0.88	31.5 sec^{-1}

The results are calculated as first-order velocity constants, each value is the average of 5 separate experiments; the standard deviation is close to 1 sec^{-1} in each case. For experimental conditions, see text. The molar ratio of spectrin to haemoglobin was 0.3 at pH 6.2, 1.1 at pH 7.15 and 0.9 at pH 7.3; the molar ratio of lysozyme to haemoglobin was 0.9.

value of $\log (pO_2)_{1/2}$ when spectrin was present. Therefore it appears that, at any given oxygen pressure, a slightly greater proportion of oxyhaemoglobin is formed as a result of the presence of spectrin.

B. Kinetic investigations. Comparative measurements have been carried out in which haemoglobin was deoxygenated either alone, or in the presence of spectrin, or another protein, lysozyme. The measurements were repeated at different pH values in the range 6–7.5. In our experimental conditions, the kinetics were pseudofirst order. (See also Salhany et al.[11].)

From inspection of table 2, it will be apparent that, at any of the 3 pH investigated, the presence of spectrin slows down the speed of the deoxygenation process of haemoglobin (while another protein, lysozyme, does not). In conclusion, kinetic as well as equilibrium studies lead one to admit that the presence of spectrin favours, to a measurable extent, the oxygenated form of haemoglobin. These experimental results suggest that an interaction between spectrin and haemoglobin can be awaited. Such an interaction is now being investigated in our laboratory.

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Central tyramine prevents hypertension in uninephrectomized DOCA-saline treated rats

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Summary. Prevention of high blood pressure in uninephrectomized, DOCA-saline treated rats was observed after treatment with central tyramine precursors. We suggest that the high blood pressure is either due to relative lack of tyrosine, which might be caused by the hyperactivity of tyrosine hydroxylase, or to hypoactivity of the decarboxylase: in both cases the result is diminished tyramine synthesis.

The rate-limiting step of catecholamine synthesis is the formation of L-DOPA from tyrosine by tyrosine hydroxylase (TH). The best known inhibitors for TH are alpha-methyl tyrosine[3], Pyratrione[4] and Oudenon[5]. All these agents decrease or prevent high blood pressure. The formation of noradrenaline from dopamine is mediated by dopamine beta hydroxylase (DBH). Inhibitors of this enzyme, such as Dopastine[5], disulfiram (Antabuse)[6] and fusaric acid[7], also decrease or prevent high blood pressure. Destruction of central adrenergic neurons by intraventricular 6-hydroxydopamine prevents the induction of hypertension[8]. Furthermore, Nagatsu et al.[9] found that NaCl administration to SH rats caused an increase in TH activity in several organs in addition to the congenitally elevated hypothalamic one[10]. Rylett et al.[11] showed that treatment with DOCA increased tyrosine hydroxylase activity and therefore increased the amount of tyrosine which enters the pathway of noradrenaline synthesis. One might therefore conclude that the inhibition of noradrenaline synthesis is responsible for the antihypertensive effect. Paradoxically, however, Lavorit and Valette[12] found that administration of tyrosine to DOCA-saline treated rats also prevents the elevation of blood pressure and speculated on the importance of central noradrenergic hypotensive centres. To eliminate the inconsistencies we hypothesized that preventing the elevation of blood pressure is due to a tyrosine metabolite not in the pathway of catecholamines' synthesis, since excess dietary tyrosine in rats does not change the catecholamine concentration in the brain[13], but still decrease the blood pressure.

Materials and methods. In this work, uninephrectomized male rats weighing 180–200 g were used. Desoxycorticosterone acetate (DOCA) 10 mg/kg was injected 3 times a week s.c. Food and saline (0.9% NaCl) were given ad libitum. Blood pressure was measured under light ether anaesthesia by a tail microphonic method[14]. For statistical analysis the Student t-test was used.

Results and discussion. From table 1 we can see that L-tyrosine 500 mg/kg totally prevents the elevation of blood pressure. The same is true for α-methyltyrosine, an inhibitor of tyrosine hydroxylase. Table 2 shows that L-tyrosine is also able to diminish existing high blood pressure. The experimental data in table 1 point to tyramine as the central hypotensive agent. This is suggested by the tyramine experiment as well as by the synergism of an ineffective dose of tyrosine with Vit. B_6 – the cofactor of amino acid decarboxylase, and the synergism of the same ineffective dose with carbidopa – a peripheral decarboxylase inhibitor. The final proof is, however, the high efficiency of D, L-tyrosine as compared with L-tyrosine[28] explained by the fact that TH is specific for the L form, but deamination shows no such specificity[15]. Finally, neither D nor L phenylalanine had any effect, indicating lack of effect of phenylethylamine. L-tryptophan is also ineffective.

Let us see how other observations fit our hypothesis. Raese et al.[16] showed that presynaptic tyrosine hydroxylase is activated by c-AMP. Dopamine inhibits that activation, therefore elevation of dopamine level, for example, by giving a precursor or inhibiting its metabolism, will inhibit tyrosine hydroxylase. DOPA indeed is

Table 1

Treatment	Intraperitoneal Daily dose mg/kg	Starting b.p.*	3 weeks b.p.*
Control	–	104 ± 3.4	155 ± 5.4, 149 ± 3.3
L-tyrosine	100	104 ± 2.8	140 ± 3.5
L-tyrosine	500	108 ± 3.8	122 ± 2.6
α-methyl-p-tyrosine	50	104 ± 2.3	124 ± 2.8
Tyramine	25	106 ± 2.6	121 ± 1.7
L-tyrosine	100	103 ± 1.6	111 ± 1.4
+vit. B_6	10		
Vit. B_6	100	105 ± 1.9	143 ± 1.9
L-tyrosine	100	110 ± 2.2	129 ± 2.3
+ carbidopa	50		
Carbidopa	50	104 ± 2.0	140 ± 2.7
D,L-tyrosine	50	104 ± 2.2	124 ± 2.5
D-phenylalanine	100	108 ± 2.5	148 ± 5.8
L-phenylalanine	500	99 ± 3.8	151 ± 6.8
L-tryptophan	500	102 ± 2.1	151 ± 6.1
p-chlorophenylalanine	50	96 ± 2.3	129 ± 3.2
D,L-dihydroxyphenylalanine	100	105 ± 2.8	162 ± 6.9

*Mean ± SE, N = 12.

known as a hypotensive agent[17]. Buckingham et al.[18] demonstrated that p-chlorophenylalanine (generally known as an inhibitor of serotonine synthesis) decreased blood pressure in DOCA-saline treated hypertensive rats. The same phenomenon was observed by Jarrott et al.[19] and by us (table 1). It seems that the decrease in blood pressure after treatment with this agent is not due to the inhibition of serotonine synthesis because 5,6-dihydroxytryptamine does not give the same result[18]. Indeed, p-chlorophenylalanine inhibits the phenylalanine (tyrosine) hydroxylase as well[20].

Our general conclusion, therefore, is that the lack of central tyramine causes high blood pressure due to lack of availability of tyrosine, increased TH activity or decreased decarboxylase efficiency. The fact that tyramine peripherically is a hypertensive agent does not detract from this argument, since by analogy L-DOPA and m-tyrosine is peripherically hypertensive, but centrally hypotensive[24]. Since c-AMP increases tyrosine hydroxylase activity[16], our scheme is in line with the supposition that increased c-AMP level might trigger hypertension. The decreased vascular c-AMP lebels are characteristic of a later phase of hypertension[23]. It is tempting to speculate that tyramine increases the free intracellular noradrenaline[21, 22] which in turn blocks tyrosine hydroxylase by negative feedbak[23] or decreases adenyl cyclase activity. The observations that in human hypertensives the plasma catecholamine concentration is linearly related to the diastolic pressure with increased capacity for noradrenaline synthesis[25], and that the inhibition of tyrosine hydroxylase by the β-receptor antagonist propranolol[26], supplement our hypothesis. Finally, in spite of the possible dopamine-tyramine conversion[27], we could not show any hypotensive action for i.p. D,L-DOPA.

Table 2. Decrease of hypertension by i.p. tyrosine during continuous DOCA-saline treatment

Treatment	Starting b.p.*	3 days b.p.*	1 week b.p.*
Control	140 ± 4.5	159 ± 5.5	165 ± 5.0
L-tyrosine 500 mg/kg daily	148 ± 5.3	116 ± 4.5	118 ± 4.6
D,L-tyrosine 100 mg/kg daily	155 ± 5.0	140 ± 6.6	125 ± 3.9

* Mean ± SE, N = 12.

1 Address for reprint requests. Part of Ph.D. thesis of B.S.
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28 Added in proof. *D*-tyrosine is even more active: 2.5 mg/kg/day.