Tyrosine Increases Blood Pressure in Hypotensive Rats

Abstract. *Administration of tyrosine, the amino acid precursor of catecholamines, increased blood pressure 38 to 49 percent in rats made acutely hypotensive by hemorrhage; other large neutral amino acids were ineffective. Tyrosine's effect was abolished by adrenalectomy, suggesting that, in hypotensive animals, it acts by accelerating the peripheral synthesis and release of catecholamines.*

Catecholamines can act at several loci to influence blood pressure. Placement of norepinephrine in the hypothalamus (*1*) or the nucleus of the solitary tract (*2*) can decrease blood pressure, presumably by diminishing sympathetic outflow. In contrast, spinal noradrenergic neurons mediate the hypertension produced in rabbits by denervation of the carotid sinus (*3*); and the release of sympathoadrenal catecholamines increases blood pressure, heart rate, and myocardial contractility (*4*).

Administration of tyrosine, or consumption of meals that elevate the ratio of plasma tyrosine to the sum of the concentrations of the other large neutral amino acids, can accelerate catecholamine synthesis in central (*5*) and peripheral (*6*) neurons, probably by increasing the saturation of the enzyme tyrosine hydroxylase (*7*). However, the extent to which any catecholaminergic neuron responds to additional tyrosine apparently varies with its firing frequency. Thus, tyrosine accelerates the release of brain norepinephrine in cold-stressed but not control rats (*8*), increases the release of dopamine from dopaminergic neurons ipsilateral to partial nigrostriatal lesions (*9*), and increases dopamine release in animals treated with haloperidol (a dopaminergic receptor blocker) (*10*) or with reserpine (*11*).

Tyrosine lowers blood pressure and increases the concentration of norepinephrine metabolites in the brainstem of hypertensive rats (*12*), suggesting that it affects catecholamine-mediated physiological functions. Furthermore, tyrosine diminishes the vulnerability of the heart to ventricular fibrillation in dogs receiving electrical currents from intracardiac electrodes (*13*). Both effects presumably result from increased noradrenergic transmission in the brainstem, leading to decreased peripheral sympathetic outflow. If tyrosine's ability to enhance norepinephrine release varies with neuronal firing rates, then tyrosine doses that lower blood pressure in hypertensive rats might raise blood pressure in hypotensive animals by enhancing the release of norepinephrine from sympathetic neurons and the adrenal medulla. This study examines the effect of tyrosine on blood pressure in rats made hypotensive by hemorrhage.

Male Sprague-Dawley rats (retired Charles River breeders weighing approximately 500 g) were anesthetized intraperitoneally with α-chloralose (50 mg/kg) and urethane ethyl carbamate (500 mg/kg) and tracheotomized. The vagus nerve and the cervical sympathetic trunk were separated from the left carotid artery and the vessel was cannulated with PE 50 tubing (*14*); blood pressure was recorded continuously through the cannula (except while intraarterial treatments were being administered) and allowed to stabilize (*15*). Hypotension was induced by bleeding the rats until 25 percent of their blood volume was lost (group 1) (*16*) or until blood pressure fell to half its starting value (group 2). The average blood pressure of the group 1 animals decreased from 106 to 63 mm-Hg. After 45 minutes, they received tyrosine (25, 50, or 100 mg/kg) or saline through the intraarterial cannula (injection time was about 5 seconds). Rats in group 2 lost 5 to 6 ml of blood; after 45 minutes, an additional milliliter was removed and replaced with an equal volume of autologous blood containing added tyrosine, saline, or another amino acid to be tested (*17*).

The administration of tyrosine to the group 1 hypotensive animals caused a dose-related increase in systolic blood pressure (Fig. 1). The 50 mg/kg dose produced a significant increase ($P < .02$) after 5 minutes; the 100 mg/kg dose produced significant increases after 5 minutes ($P < .001$), 15 minutes ($P < .02$), and 30 minutes ($P < .02$) (Fig. 1). Tyrosine also significantly increased blood pressure in the group 2 rats ($P < .002$).

To evaluate the adrenals' contribution to the effect of tyrosine on blood pressure, we assessed its effect in rats subjected to hemorrhage alone or hemorrhage plus bilateral adrenalectomy. One hour after bilateral adrenalectomy (*18*), animals were bled (1 to 2 ml) to cause a 50 percent reduction in blood pressure and were given tyrosine (100 mg/kg) 45 minutes later. In the adrenalectomized animals tyrosine did not cause a significant increase in blood pressure, whereas in rats bled but not adrenalectomized it caused a 38 percent increase (Table 1). These results suggest that catecholamine-producing cells of the adrenal medulla are the major site of tyrosine activity in hypotensive rats.

We also examined the effects of three other large neutral amino acids on blood pressure in hypotensive rats. [These compounds share a common transport system with tyrosine for uptake into the brain (*19*) but are not themselves substrates for any of the enzymes that convert tyrosine to catecholamines or metabolize catecholamines.] Administration of 100 mg of valine or leucine per kilogram failed to affect blood pressure (Table 1), while tryptophan killed four of the seven animals tested and did not produce any significant change in blood pressure among the surviving three.

These observations indicate that tyrosine can raise blood pressure in acutely hypotensive rats and suggest that tyrosine acts by enhancing the peripheral synthesis and release of catecholamines. That a given dose of tyrosine can either

Table 1. Effects of tyrosine, tyrosine plus adrenalectomy, and other large neutral amino acids (100 mg/kg) on systolic blood pressure in the group 2 hypotensive rats 5 minutes after treatment.

Treatment	*N*	Increase in blood pressure (%)
Tyrosine	12	38 ± 10*
Tyrosine plus adrenalectomy	6	13 ± 7
Saline	12	11 ± 3
Valine	10	8 ± 5
Leucine	8	3 ± 1
Tryptophan	7†	17 ± 4

*Significantly different from the increase for the group receiving tyrosine plus adrenalectomy ($P < .03$, analysis of variance) and from the increase for the saline-treated group ($P < .002$). †Four died; value is for the survivors.

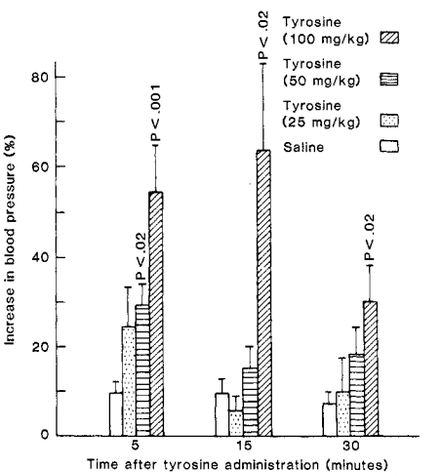

Fig. 1. Increase in systolic blood pressure in the group 1 hypotensive rats 5, 15, and 30 minutes after they were given various doses of tyrosine or saline. Statistical comparisons are with the increases for the saline-treated rats.

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raise (Fig. 1 and Table 1) or lower (*12*) blood pressure, depending on the animal's starting blood pressure, affirms the hypothesis that the ability of tyrosine to accelerate catecholamine synthesis depends on the firing frequency of catecholamine-producing cells. In hypertensive rats, it seems likely that noradrenergic neurons in the brainstem fire frequently and that sympathetic outflow from more peripheral neurons is diminished (*12*); in hypotensive animals, this situation is probably reversed. If tyrosine exerts a similar state-dependent effect on blood pressure in humans, it may have advantages over catecholamine infusions in the treatment of shock.

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14. Heparin (400 U/g) was then administered intraarterially.
15. Intraarterial blood pressure was recorded with a Grass model 70 polygraph and Statham transducers P23DC and P23AC.
16. Blood volume was estimated as 55 ml/kg [H. Donaldson, *The Rat* (Wistar Institute, Philadelphia, 1924)].
17. The other amino acids were L-tyrosine methylester HCl, L-tryptophan methylester HCl, L-valine, and L-leucine; all solutions were adjusted to *p*H 5.0.
18. One hour after adrenalectomy, blood pressure had not changed significantly ($P < .2$).
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20. Supported in part by NIH grant AM-14228, NASA grant NGR-22-009-627, the Center for Brain Sciences, and the Metabolism Charitable Trust. L.A.C. is supported by National Research Training Award GM-07592.

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Cadmium-113 Nuclear Magnetic Resonance Studies of Bovine Insulin: Two-Zinc Insulin Hexamer Specifically Binds Calcium

Abstract. *By use of cadmium-113 nuclear magnetic resonance spectroscopy, a specific calcium ion binding site has been identified in the bovine two-zinc insulin hexamer. This site is composed of six glutamyl carboxylate groups clustered in the center of the hexamer, and is distinct from the normal zinc ion binding sites.*

We present here results of ^{113}Cd nuclear magnetic resonance (NMR) experiments which provide evidence that Ca^{2+} binds strongly to a specific site in the central core of the bovine two-zinc insulin hexamer. The Ca^{2+} site is composed of six glutamyl carboxylate groups (Glu B-13) clustered in the center of the hexamer, and is distinct from the normal Zn^{2+} binding sites (*1*). X-ray crystallographic studies of porcine zinc insulin by Blundell *et al.* (*2*) showed that the hexamer is torus-shaped (~ 50 Å in diameter by 35 Å high). The two Zn^{2+} ions per hexamer are located 17 Å apart on the threefold symmetry axis, which traverses the central cavity. Each Zn^{2+} ion is coordinated to three imidazolyl nitrogen atoms (from His B-10) and three water molecules (see Fig. 1). The crystallographically identified Zn^{2+} sites (*2*) are designated site I, and the Ca^{2+} site proposed here is designated site II (Fig. 1).

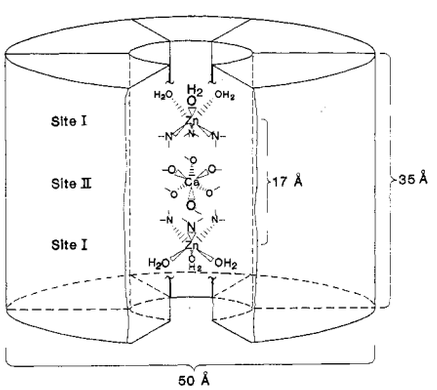

Fig. 1. Three-dimensional structural representation of the zinc insulin hexamer, showing the proposed Ca^{2+} binding site (site II). Ligation about Zn^{2+} ions (3 His, 3 H_2O, site I) is based on the x-ray crystal structure (*1*). The experiments represented in Fig. 2 are consistent with a single site for Ca^{2+}, but do not exclude exchange among several identical sites (that is, sites composed by pairing the carboxylates). The insulin monomer facing the viewer has been cut away to show the positions and liganding of the metal ion sites.

Synthesized in the beta cells of the pancreatic islets of Langerhans, insulin is stored in secretory granules. Under the electron microscope, immature granules appear to contain insulin in an amorphous state. In mature granules, insulin forms a dense array of 50-Å units, with packing similar to that found in the crystalline hexamer (*3*). Because of the high content of zinc in pancreatic islets and the requirement for divalent metals in forming rhombohedral crystals, it has been proposed that the two-zinc insulin hexamer, $(Zn^{2+})_2(Ins)_6$, is the storage form of insulin (*2*).

Substitution of $^{113}Cd^{2+}$ ions for naturally occurring divalent metal ions and direct observation of the ^{113}Cd NMR signal has proved useful in the study of metalloproteins (*4–7*). Cadmium-113 chemical shifts depend strongly on the number and type of ligands coordinated to the metal ion.

The ^{113}Cd NMR spectrum of ^{113}Cd-substituted bovine insulin hexamer at *p*H 8.0 (Fig. 2A) exhibits two resonances of relative area 2:1. Peak I has a chemical shift of 165 ppm and peak II occurs at −36 ppm. When $^{113}Cd^{2+}$ is added in small increments from an initial ratio of 0.3 equivalent per hexamer to a final ratio of 3.0, the resulting ^{113}Cd NMR spectra (not shown) have the same chemical shifts and 2:1 relative area. When this species, which is designated $(^{113}Cd^{2+})_3(Ins)_6$, was titrated from *p*H 8.0 to *p*H 10.4 by gradual addition of NaOH, peak I underwent a downfield shift, which leveled off at 201 ppm. Peak II was unaffected. Least-squares fitting of peak I chemical shifts versus *p*H to a sigmoidal curve gave an apparent pK_a value of 8.7. We propose that this *p*H dependence is caused by conversion of metal-bound water to hydroxide ion (*8*). Gradual addition of NaCl produced a

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