Inhibitory Action of Hydralazine on Catecholamine-Synthesizing Enzymes Prepared from Bovine Adrenal Medulla

Kyoji MORITA, Hitoshi HOUCHI, Atsushi NAKANISHI, Kazuo MINAKUCHI and Motoo OKA
Department of Pharmacology, Tokushima University School of Medicine, Kuramoto, Tokushima 770, Japan

Accepted December 9, 1985

Abstract—The direct effect of hydralazine on catecholamine-synthesizing enzymes was investigated. Hydralazine caused a concentration-dependent inhibition of tyrosine hydroxylase (TH) prepared from bovine adrenal medulla, and a more pronounced effect was obtained by incubating the enzyme with the drug prior to the enzyme assay. Kinetic studies showed that hydralazine increased the apparent Km value of the enzyme for tyrosine and cofactor, 6,7-dimethyl-5,6,7,8-tetrahydropterin (DMPH₄), without any change in the Vmax. The inhibitory effect of the drug was irreversible, and an excess amount of FeSO₄ failed to restore the enzyme activity inhibited by this drug. Furthermore, hydralazine also inhibited the dopamine β-hydroxylase (DBH) in chromaffin granule membranes. Hydralazine increased the apparent Km value of DBH for ascorbic acid without any change in the Vmax, and it decreased the Vmax of the enzyme for tyramine with no change in the apparent Km value. The observations described here suggest the possibility that hydralazine presumably causes the inhibition of catecholamine-synthesizing enzymes as a result of allosteric alterations in the molecular structures of these enzymes. It thus seems unlikely that the inhibitory action of hydralazine on these enzymes may totally be based on its metal-chelating activity.

Hydralazine (1-hydrazinophthalazine) is generally known to be a potent antihypertensive drug and commonly used for the treatment of hypertension. The antihypertensive action of hydralazine is thought to be due to a decrease in the peripheral resistance of the circulating system as a result of the dilation of blood vessels. However, the detailed mechanism of the vasodilating action of this drug is still unclear. As a possible mechanism of the hypotensive action, the direct effect of hydralazine on the vascular system has been suggested. It has been shown that hydralazine specifically inhibits thromboxane synthetase and possibly causes the dilation of blood vessels as a result of reducing the production of an endogenous vasoconstrictor, thromboxane A₂ (1). Recent studies have shown that hydralazine can directly act upon vascular smooth muscle and results in the inhibition of calcium-dependent ATPase and protein phosphorylation in isolated myofibrils. These results therefore seem to indicate the possibility that the vasodilating action of hydralazine may be due to the direct action of the drug on the contractile apparatus of vascular smooth muscle (2, 3).

Hydralazine has already been shown to cause an early depletion of catecholamines from several organs in vivo (4–6). Furthermore, Chevillard et al. have shown that the overflow of norepinephrine from the segments of rat tail artery is markedly depressed by hydralazine (7). These observations therefore seem to indicate the possibility that hydralazine may induce the vasodilation through the modulation of sympathetic nerve activity. On the other hand, hydralazine has been reported to have a certain effect on the metabolism of biogenic amines in vivo. It has been reported that the chronic adminis-
tation of hydralazine causes an alteration in the activities of catecholamine-synthesizing enzymes, tyrosine hydroxylase (TH, EC 1.14.16.2) and dopamine β-hydroxylase (DBH, EC 1.14.17.1), in spontaneous hypertensive rats (8, 9). Furthermore, hydralazine has been found to inhibit the conversion of dopamine to norepinephrine (10) as a result of the inhibition of DBH (11). Persson et al. have also reported that the accumulation of DOPA in rat brain is reduced by the administration of dihydralazine and therefore suggested the possibility that the biosynthesis of catecholamines may be suppressed by the drug as a result of the inhibition of TH (12).

In addition to the studies on the enzymes involved in catecholamine biosynthesis, the inhibitory effect of hydralazine on monoamine oxidase has also been reported (13, 14).

In the present work, we have investigated the direct action of hydralazine on TH and DBH prepared from bovine adrenal medulla and reported here the properties of the inhibitory action of the drug on these enzymes.

Materials and Methods

Enzyme preparation: Fresh bovine adrenal medulla was homogenized with ice-cold 0.3 M sucrose containing 10 mM Tris-HCl (pH 7.4). The homogenate was filtered through 4-layers of gauze and then centrifuged at 600×g for 10 min. The obtained supernatant fraction was centrifuged at 12,000×g for 30 min to separate the large granule fraction. The post-mitochondrial fraction was recentrifuged at 105,000×g for 60 min, and the resulting supernatant fraction was dialyzed against 20 mM Tris-acetate (pH 6.0). The dialyzed soluble fraction was stored in small aliquots at −80°C and used for the determination of TH activity. Chromaffin granules were prepared from the large granule fraction. The 12,000×g pellet was resuspended in 0.3 M sucrose buffered with 10 mM Tris-HCl (pH 7.4), and a 5 ml-aliquot of the suspension was overlayered on a 20 ml-layer of 1.6 M sucrose containing 10 mM Tris-HCl (pH 7.4). After centrifuging at 105,000×g for 1 hr, chromaffin granules precipitated at the bottom of the tube were resuspended in 20 mM Tris-acetate (pH 6.0) and then stored frozen overnight. The frozen suspension was thawed and centrifuged at 30,000×g for 30 min. The pellets of chromaffin granule membranes were washed 3 times with 20 mM Tris-acetate (pH 6.0), and the suspension of granule membranes was dialyzed against the same buffer. Obtained granule membranes were used as the enzyme preparation in the experiments of DBH.

Determination of enzyme activity: TH activity in the soluble fraction was determined according to the method described by Yamauchi and Fujisawa (15) with some modifications. The mixture containing 200 mM Tris-acetate (pH 6.0), 100 µg of catalase, 100 mM 2-mercaptoethanol, 1 mM DMPH₄, 0.2 mM L-tyrosine and the soluble fraction in a final volume of 0.5 ml was incubated at 37°C for 10 min, and the reaction was terminated by adding 2 ml of 0.5 N perchloric acid. DOPA formed during the incubation period was then isolated by the aluminum hydroxide-gel adsorption method (16, 17) and determined by the modified trihydroxyindole method using potassium ferricyanide (18). The oxidation reaction was carried out at pH 7.0 for 20 min on ice and stopped by adding alkaline ascorbate solution. After standing for 30 min at room temperature, fluorescence intensity was determined at 360 nm (excitation)-490 nm (emission).

DBH activity in chromaffin granule membranes was measured by the spectrophotometric method using tyramine as a substrate (19, 20). The mixture containing 200 mM Na-acetate (pH 5.5), 50 µg of catalase, 10 mM fumarate, 10 mM N-ethyl maleimide, 10 mM tyramine, 10 mM ascorbic acid and the granule membranes in a final volume of 1 ml was incubated at 37°C for 10 min. Octopamine formed during the reaction period was isolated by an ion-exchange column and then determined by measuring the absorbance at 330 nm after converting it to p-hydroxybenzaldehyde by periodate oxidation.

A pair of tubes containing a complete reaction mixture were prepared for a blank and an internal standard. One of them was kept on ice as the enzyme blank, and the other tube containing a certain amount of DOPA (for TH assay) or octopamine (for
DBH assay) as the internal standard was incubated. These tubes were carried through the entire assay procedure. A linear increase in the activities of both TH and DBH as a function of the time of incubation was observed up to at least 30 min. Neither spectrofluorometric assay of DOPA nor spectrophotometric assay of octopamine interfered with the drugs at the concentrations used in these experiments.

**Chemicals:** Hydralazine hydrochloride (Apresoline) was kindly donated by Kyowa Fermentation Industry Co. (Tokyo, Japan). N-ethylmaleimide, L-DOPA, DL-octopamine hydrochloride, fusaric acid and catalase were purchased from Sigma Chemical Co. Tyramine hydrochloride was obtained from Merck. DMPPH₄ was obtained from Aldrich Chemical Co. Other chemicals were of commercially available reagent grade. The reagents were neutralized if it was necessary.

**Results**

The effect of hydralazine on the activity of TH in the soluble fraction of bovine adrenal medulla was examined. As shown in Fig. 1, the concentration-dependent inhibitory effect of hydralazine on TH was observed at the concentrations above $10^{-4}$ M, and 50% inhibition of the enzyme was obtained by adding about $10^{-3}$ M of hydralazine. The effects of Fe²⁺-chelating agents on the enzyme activity were examined for comparison. The Fe²⁺-chelating agents $a,a'$-dipyridyl and o-phenanthroline had an inhibitory effect on TH, and 50% inhibition of the enzyme was obtained at about $10^{-4}$ M and $10^{-6}$ M, respectively (Fig. 1). These chelating agents were shown to be more effective than hydralazine as an inhibitor of TH in vitro. A more pronounced inhibitory effect of hydralazine on TH was obtained by incubating the enzyme with the drug prior to the assay reaction. As shown in Fig. 2, the inhibitory effect of hydralazine on TH activity markedly increased according to the preincubation time, but no significant change in the activity of the control enzyme was observed during the preincubation period (data not shown).

The results of kinetic studies are shown in Fig. 3A and 3B. After preincubating the
enzyme at 37°C for 15 min with or without 10^{-4} M of hydralazine, the enzyme activity was determined in the presence of the various concentrations of the cofactor DMPH4 (0.2-1 mM) or the substrate tyrosine (0.04-0.2 mM). Kinetic analyses showed that hydralazine caused a significant increase in the apparent $K_m$ value of the enzyme for both DMPH4 (Fig. 3A) and tyrosine (Fig. 3B), without any change in the $V_{max}$. The effect of hydralazine on DBH, another important enzyme in the biosynthetic pathway.

Further studies were carried out to examine whether the effect of hydralazine on TH activity was reversible or not. The mixture containing TH and 10^{-3} M of hydralazine was incubated at 4°C or 37°C for 15 min and diluted with the buffer to lower the drug concentration in the assay mixture below the effective range as an inhibitor of TH (approximately 10^{-5} M as shown in Fig. 1), and the enzyme activity was then determined. As shown in Table 1, the drug brought into the assay system from the mixture preincubated at 4°C did not cause any difference in the enzyme activity between the control and the treated enzyme. On the other hand, the preincubation of the enzyme with the drug at 37°C markedly decreased the enzyme activity by approximately 25% of the control, and there was no recovery of the enzyme inhibited by the drug after dilution of the mixture. Furthermore, the effect of ferrous ion on the inhibitory action of hydralazine was investigated. The enzyme was preincubated with hydralazine and the enzyme activity was determined in the presence or absence of 1 mM FeSO4. The results in Table 2 showed that the addition of FeSO4 failed to restore the enzyme activity inhibited by the drug during the preincubation period.

The effect of hydralazine on DBH, another important enzyme in the biosynthetic pathway...
Table 1. Reversibility of the inhibitory effect of hydralazine on soluble TH prepared from bovine adrenal medulla

| Preincubation  | Soluble TH activity (nmol DOPA/mg protein/10 min) |
|---------------|--------------------------------------------------|
| None          | 46.4±3.1                                         |
| Hydralazine   | 47.1±6.1                                         |

The enzyme preparation was preincubated with or without $10^{-3}$ M of hydralazine at 4°C or 37°C for 15 min in 200 mM Tris-acetate (pH 6.0), and the mixture was then diluted with the same buffer. The enzyme activity in the aliquot of diluted mixture was determined as described in the text. The final concentration of the drug in the assay mixture was approximately $10^{-6}$ M. Values are the mean±S.D. of three experiments with triplicate determinations.

Table 2. Effect of FeSO$_4$ on the inhibitory action of hydralazine on soluble TH prepared from bovine adrenal medulla

| Hydralazine | Soluble TH activity (FeSO$_4$ (-), nmol DOPA/mg protein/10 min) | Soluble TH activity (FeSO$_4$ (+), nmol DOPA/mg protein/10 min) |
|------------|------------------------------------------------------------------|-----------------------------------------------------------------|
| 0 M        | 42.7±7.0 (0)                                                     | 43.8±2.4 (0)                                                   |
| $10^{-5}$ M | 40.0±2.4 (6)                                                     | 42.3±7.8 (3)                                                   |
| $10^{-4}$ M | 23.6±4.5 (45)                                                   | 27.8±3.1 (37)                                                  |
| $10^{-3}$ M | 9.4±4.4 (78)                                                     | 5.0±1.7 (89)                                                   |

The enzyme preparation was preincubated at 37°C for 15 min with various concentrations of hydralazine in 200 mM Tris-acetate (pH 6.0), and the enzyme activity was then determined in the presence and absence of 1 mM FeSO$_4$ as described in the text. The drug concentration was kept constant in both the preincubation and the assay mixture. Results are expressed as the mean±S.D. of three experiments with triplicate determinations. Values in parentheses are the inhibition percent.

of catecholamines, was investigated using chromaffin granule membranes as an enzyme preparation. As shown in Fig. 4, hydralazine inhibited DBH at the concentration of $10^{-6}$ M or higher, but it was approximately 100-fold less effective than Cu$^{2+}$-chelating agents, diethylidithiocarbamate and fusaric acid, as an inhibitor of the enzyme.

Lineweaver-Burk analyses indicated that hydralazine caused a marked increase in the apparent $K_m$ value of DBH for ascorbic acid with no change in the $V_{max}$ (Fig. 5A). By contrast, no change in the apparent $K_m$ value of DBH for tyramine was observed, but a significant decrease in the $V_{max}$ was observed by the addition of the drug (Fig. 5B).

To examine whether the inhibitory effect of hydralazine on TH and DBH was the specific action of this drug or not, the effects of other hydrazine-based compounds on these enzymes were studied. As shown in Table 3, hydrazine itself affected neither TH nor DBH activity; and the antituberculous drug isoniazid also had no effect on both enzymes. Only hydralazine, which is the derivative of phthalazine with a hydrazine-group, had inhibitory action on both TH and DBH under the experimental conditions used here.

**Discussion**

The considerable evidence for suggesting that hydralazine may have a significant influence on catecholamine biosynthesis has already been presented (8–11). The inhibition of DBH by this drug has been shown either in intact tissue (10) or in isolated enzyme
However, there is no evidence for indicating the inhibitory action of hydralazine on TH, except indirect evidence that the administration of the drug causes a decrease in the accumulation of DOPA in rat brain (12). Therefore, we studied the direct action of hydralazine on the activity of TH prepared from bovine adrenal medulla and found that the enzyme was inhibited by the addition of hydralazine (Fig. 1). The inhibitory action of hydralazine was observed at the relatively higher concentrations, but the effect of this drug became more pronounced by incubating the enzyme with the drug prior to the assay reaction (Fig. 2). This fact suggests that hydralazine seems to cause the inhibitory effect on TH as a result of direct action on the enzyme itself. In consequence of the interaction between TH and hydralazine, an increase in the apparent $K_m$ value of the enzyme for both tyrosine and DMPH$_4$ was observed (Fig. 3A and 3B). It is therefore conceivable that this drug may cause an alteration of the enzyme structure and result

Fig. 4. Inhibitory effects of hydralazine and Cu$^{2+}$-chelating agents on DBH activity in chromaffin granule membranes prepared from bovine adrenal medulla. The granule membranes were incubated at 37°C for 10 min in the presence of various concentrations of hydralazine (●), fusaric acid (○) and diethyldithiocarbamate (△). Octopamine formed during the reaction was determined as described in the text. Each point is the mean of three experiments with triplicate determinations.

Fig. 5. Influence of hydralazine on the kinetic properties of DBH. A: Effect of hydralazine on the apparent $K_m$ value of DBH for cofactor, ascorbic acid. The enzyme activity was determined in the presence (●) and absence (○) of 5×10⁻⁸ M hydralazine, using 10 mM tyramine and various concentrations of ascorbic acid, as described in the text. Velocity of the reaction is expressed as μmoles octopamine formed/mg protein/10 min. Each point is the mean of three experiments with duplicate determinations. B: Effect of hydralazine on the $V_{max}$ of DBH for substrate, tyramine. The enzyme activity was measured in the presence (●) and absence (○) of 5×10⁻⁸ M hydralazine, using 10 mM ascorbic acid and various concentrations of tyramine, as described in the text. Velocity of the reaction is expressed as μmoles octopamine formed/mg protein/10 min. Each point is the mean of three experiments with duplicate determinations.
in a decrease in the affinity of the enzyme for both substrate and cofactor.

In the treatment of hypertension, hydralazine is usually used continuously for a long period. Therefore, it seemed important to test whether the inhibitory action of the drug was reversible or not. Although the concentration of the drug in the assay mixture was lowered below the effective level, the inhibitory effect of hydralazine on the enzyme was still retained as it was (Table 1). Furthermore, it seemed interesting to examine the question of whether the inhibition of the enzyme by hydralazine was restored by adding FeSO₄, since hydralazine has strong activity as a metal-chelating agent. As shown in Table 2, the addition of FeSO₄ did not show any significant ability to restore the activity of the enzyme inhibited by the drug. These results suggest that hydralazine may cause the irreversible inhibition of TH, and the inhibitory action of this drug may not be due to the removal of iron from the enzyme molecule. On the other hand, Hoeldtke and Kaufman (21) have previously made an attempt to separate protein-bound iron from the TH molecule by the treatment with various metal-chelating agents, α,α'-dipyridyl, o-phenanthroline or Chelex 100, and reported that their attempts were not successful. They have also reported that partial resolution of protein-bound iron was accomplished by treatment of the enzyme with ammonium sulfate followed by chromatography on Sephadex G-25 and that a loss of the enzyme activity, which could be restored by FeSO₄, was obtained by this procedure. In view of these findings, it seems not always reasonable to consider that the inhibitory effect of the drugs, even though they have an ability to form a chelating complex with iron, may be due to a removal of the iron from the enzyme. Thus, our observations described here seem to indicate that the inhibitory action of hydralazine on TH may be due to an irreversible change in the structure of the enzyme molecule which may result in a decrease in the affinity of the enzyme for both substrate and cofactor, and they also suggest the possibility that the drug may cause the inhibitory action on TH through a mechanism different from the removal of iron from the enzyme molecule.

Hydralazine has already been reported to cause the inhibition of DBH through the chelation of Cu²⁺, which was thought to be required for the inactivation of an endogenous inhibitor (12). We therefore examined the effect of hydralazine on DBH associated to chromaffin granule membranes prepared from bovine adrenal medulla and confirmed the inhibitory action of this drug on DBH (Fig. 5). Although DBH activity was determined in the mixture containing an excess amount of N-ethylmaleimide to inactivate the endogenous inhibitory SH-groups, the inhibitory action of

Table 3. Effects of hydrazine-based compounds on soluble TH and granule membrane DBH prepared from bovine adrenal medulla

| Hydrazine-based compounds | Soluble TH activity¹ | Membrane DBH activity¹ |
|--------------------------|----------------------|------------------------|
|                          | n mol DOPA/mg protein/10 min | µ mol Octopamine/mg protein/10 min |
| None                     | 42.4±4.5              | 3.16±0.24              |
| Hydralazine, 10⁻⁴ M      | 19.6±1.5              | 0.63±0.04              |
| 10⁻³ M                   | 8.3±0.6               |                        |
| Hydrazine, 10⁻⁴ M        | 47.2±2.6              | 3.25±0.37              |
| 10⁻³ M                   | 46.3±4.0              |                        |
| Isoniazid, 10⁻⁴ M        | 43.3±6.0              | 3.18±0.15              |
| 10⁻³ M                   | 44.0±1.7              |                        |

¹Soluble TH activity was determined as described in the text after preincubating the enzyme preparation with hydrazine-based compounds at 37°C for 15 min. Membrane DBH activity was measured as described in the text in the presence of hydrazine-based compounds without preincubation. Values are the mean±S.D. of three experiments with triplicate determinations.
the drug was still observed under these conditions. Hydralazine therefore seems to inhibit DBH as a result of chelating the protein-bound Cu²⁺ which is required for the catalytic activity of the enzyme rather than for the inactivation of endogenous inhibitor.

The inhibitory action of hydralazine on DBH was furthermore shown to be competitive with ascorbic acid and noncompetitive with tyramine (Fig. 5A and 5B). These results seem to indicate that the drug may primarily act at the ascorbic acid-acting site in the DBH molecule and probably causes an apparent decrease in the number of active enzyme molecules, which is reflected by a decrease in the \( V_{\text{max}} \) for the substrate. According to the proposed mechanism of the hydroxylation reaction, the protein-bound copper in the enzyme is first reduced by ascorbic acid, and consequently the enzyme becomes capable of hydroxylating the substrate (22, 23). It therefore seems conceivable that hydralazine may primarily act on the protein-bound copper as a metal-chelating agent and prevent the reduction of copper by ascorbic acid on the enzyme molecule, thus causing the inhibition of the enzyme as a result of blocking the regeneration of the reduced-form active enzyme.

In addition, other hydrazine-based compounds were also shown to affect neither TH nor DBH, although hydralazine could inhibit these two enzymes (Table 3). These results seem to indicate that the inhibitory action of hydralazine on TH and DBH may be due to the intrinsic activity of the drug itself, but not related to the chemical activity of the hydrazine-group.

In the present studies, we have investigated the direct action of hydralazine on catecholamine-synthesizing enzymes, and we found that hydralazine can inhibit both TH and DBH prepared from bovine adrenal medulla. The concentrations of hydralazine required for the inhibition of these enzymes were shown to be probably higher than the tissue concentration that patients receiving the drug would be expected to have. Thus, it is thought to be uncertain whether hydralazine may actually have an inhibitory action on these enzymes in vivo. However, the inhibitory action of hydralazine was shown to be competitive with tyrosine and DMPH₄ for TH and with ascorbic acid for DBH. It therefore seems reasonable to assume that higher concentration of hydralazine is presumably required for exhibiting the inhibitory action in the presence of saturating concentrations of substrate and cofactor. In view of the fact that the concentrations of pterin cofactor and ascorbic acid are not high enough to saturate TH and DBH within the cell, it seems conceivable that hydralazine may be able to cause the inhibition of these enzymes within the cell at concentrations lower than that estimated under our experimental conditions.

Although the physiological meaning of the inhibitory action of hydralazine on catecholamine-synthesizing enzymes, particularly the significance of this effect in the mechanism of antihypertensive action, was not completely elucidated, our findings suggest the possibility that hydralazine presumably causes a decrease in the blood concentration of catecholamines as well as the depletion of neurotransmitter in sympathetic nerve terminals, thus lowering the blood pressure in hypertensive patients.

References
1 Luderer, J.R., Demers, L.M., Janson, R.W., Nomides, C.T. and Hayes, A.H., Jr.: The effect of hydralazine on arachidonic acid metabolism in isolated, washed human platelets. Res. Commun. Chem. Pathol. Pharmacol. 28, 43–52 (1980)
2 Seidel, C.L., Allen, J.C. and Bowers, R.L.: Mechanical and biochemical alterations of aorta induced by hydralazine hypotension. J. Pharmacol. Exp. Ther. 213, 514–519 (1980)
3 Jacobs, M.: Mechanism of action of hydralazine on vascular smooth muscle. Biochem. Pharmacol. 33, 2915–2920 (1984)
4 Bydgeman, M. and Stjärne, L.: Effects of 1,4-dihydrazinophthalazine on organ content and adrenal medullary secretion of catecholamines. Nature 184, 1646–1647 (1959)
5 Bydgeman, M. and Stjärne, L.: Effects of 1,4-dihydrazinophthalazine on the catecholamines. Nature 186, 82–83 (1960)
6 Linet, O., Van Zwieten, P.A. and Hertting, G.: Effects of hydrazinophthalazines on catecholamines in rats. Eur. J. Pharmacol. 6, 121–124 (1969)
7 Chevillard, C., Mathieu, M.N., Saiag, B. and
Worcel, M.: Hydralazine: Effect on the outflow of noradrenaline and mechanical responses evoked by sympathectic nerve stimulation of the rat artery. Br. J. Pharmacol. 69, 415–420 (1980)

8 Kohler, C., Berkowitz, B.A. and Spector, S.: Antihypertensive drugs and catecholamine metabolism: Effects of reserpine and hydralazine on tyrosine hydroxylase activity and norepinephrine concentrations in the spontaneously hypertensive rat. J. Pharmacol. Exp. Ther. 193, 443–451 (1975)

9 Denoroy, L., Renaud, B., Vincent, M., Sacquet, J. and Sassard, J.: Dihydralazine and catecholamines-synthesizing enzymes in spontaneous hypertension. Eur. J. Pharmacol. 58, 207–210 (1979)

10 Songkittiguna, P., Majewski, H. and Rand, M.J.: Inhibition by hydralazine of the conversion of dopamine to noradrenaline in rat atria in vitro and in vivo. Clin. Exp. Pharmacol. Physiol. 7, 509–514 (1980)

11 Liu, T.Z., Shen, J.-T. and Loken, T.F.: Inhibition of dopamine-β-hydroxylase by hydralazine. Proc. Soc. Exp. Biol. Med. 145, 294–297 (1974)

12 Persson, B., Svensson, T.H. and Henning, M.: Dihydralazine inhibits tyrosine hydroxylase in vivo in the rat. J. Neural Transm. 53, 109–116 (1982)

13 Lyles, G.A. and Callingham, B.A.: Hydralazine is an irreversible inhibitor of the semicarbazide-sensitive, clorgyline-resistant amine oxidase in rat aorta homogenates. J. Pharm. Pharmacol. 34, 139–140 (1982)

14 Lyles, G.A., Garcia-Rodriguez, J. and Callingham B.A.: Inhibitory actions of hydralazine upon monoamine oxidizing enzymes in the rat. Biochem. Pharmacol. 32, 2515–2522 (1983)

15 Yamauchi, T. and Fujisawa, H.: A simple and sensitive fluorometric assay for tyrosine hydroxylase. Anal. Biochem. 89, 143–150 (1978)

16 Von Euler, U.S.: Preparation, purification and evaluation of noradrenaline and adrenaline in organ extracts. Arch. Int. Pharmacodyn. Ther. 77, 477–485 (1948)

17 Burn, G.P. and Fild, E.O.: Colorimetric method for diagnosis of pheochromocytoma. Br. Med. J. 2, 1152–1154 (1956)

18 Morita, K., Brocklehurst, K.W., Tomares, S.M. and Pollard, H.B.: The phorbol ester TPA enhances A23187- but not carbachol- and high K+-induced catecholamine secretion from cultured bovine adrenal chromaffin cells. Biochem. Biophys. Res. Commun. 129, 511–516 (1985)

19 Pisano, J.J., Creveling, C.R. and Udenfriend, S.: Enzymic conversion of p-tyramine to p-hydroxyphenylethanolamine (norsynephrine). Biochim. Biophys. Acta 43, 566–569 (1960)

20 Creveling, C.R., Daly, J.W., Witkop, B. and Udenfriend, S.: Substrates and inhibitors of dopamine-β-hydroxylase. Biochim. Biophys. Acta 64, 125–134 (1962)

21 Hoeldtke, R. and Kaufman, S.: Bovine adrenal tyrosine hydroxylase: Purification and properties. J. Biol. Chem. 252, 3160–3169 (1977)

22 Friedman, S. and Kaufman, S.: 3,4-Dihydroxyphenylethylamine β-hydroxylase physical properties, copper content, and role of copper in the catalytic activity. J. Biol. Chem. 240, 4763–4773 (1965)

23 Goldstein, M., Joh, T.H. and Gravey, T.Q., III.: Kinetic studies of the enzymatic dopamine β-hydroxylation reaction. Biochemistry 7, 2724–2730 (1968)