INHIBITION OF DOPAMINE $\beta$-HYDROXYLASE IN BLOOD VESSELS BY PICOLINIC ACID DERIVATIVES IN VIVO AND THEIR ANTIHYPERTENSIVE EFFECTS

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Abstract—The effect of picolinic acid derivatives, 5-butylpicolinic (fusaric) acid (FA), 5-(3',4'-dibromobutyl)picolinic acid (Br$_2$FA) and 5-(N,N-dimethyldithiocarbamoilmethyl) picolinic acid (YP-279) on dopamine $\beta$-hydroxylase in blood vessels in vivo was studied. Maximum inhibition of the conversion of $^{14}$C-dopamine ($^{14}$C-DA) to $^{14}$C-norepinephrine ($^{14}$C-NE) in rat aorta, mesenteric artery and renal artery was detected 30 min after FA and Br$_2$FA (75 mg/kg) and 60 min after YP-279 (75 mg/kg). NE synthesis from $^{14}$C-DA returned to near control values by 6 hr in the blood vessels. NE levels of the aorta and mesenteric artery were significantly reduced by 30 to 50% at 4 hr after Br$_2$FA or FA (75 mg/kg). Dopamine $\beta$-hydroxylase (DBH) activity, using tyramine as substrate, in heart, aorta, mesenteric artery and renal artery was markedly reduced. The concentrations of FA, Br$_2$FA and YP-279 in rat blood following a single i.p. injection of each drug increase rapidly, reaching highest values in 0 to 30 min and decreasing slowly to 0 after 6 hr. These compounds did not affect the uptake of $^3$H-NE into the rat heart. These three compounds were found to lower blood pressure effectively in normal Wistar rats (above 25 mg/kg).

In studying a large number of picolinic acid derivatives, several compounds, 5-butylpicolinic acid (FA), 5-(3', 4'-dibromobutyl) picolinic acid (Br$_2$FA) and 5-(N,N-dimethyldithiocarbamoilmethyl) picolinic acid (YP-279) have been found in vitro to be the most potent inhibitors of dopamine $\beta$-hydroxylase [3',4-dihydroxyphenylethylamine, ascorbate: oxygen oxidoreductase (hydroxylating), EC 1.14.2.1.] (DBH). These compounds produced 50% inhibition at concentrations of $1 \times 10^{-8}$ M, $7.7 \times 10^{-8}$ M and $7.6 \times 10^{-8}$ M respectively (1). Endogenous levels of norepinephrine (NE) in the heart and epinephrine (E) in the adrenals of SHR (spontaneously hypertensive rats) were found to be reduced after a single 50 mg/kg dose of either FA or Br$_2$FA (2).

A significant reduction of blood pressure in normotensive and hypertensive man was observed after administration of a single 6 mg/kg p.o. dose of 5-butylpicolinic acid calcium salt (FA-Ca) (3, 4). Peak plasma level of FA-Ca was detected 0.5–1 hr after drug administration. The decrease in blood pressure also was demonstrated in SHR and the normotensive rabbit after the i.v. administration of 50 mg/kg of FA and Br$_2$FA (2). Moreover, in a comparative study of several 5-alkylpicolinic acids, Suda et al (5) reported that reduction of blood pressure was more marked when a more potent inhibitor of DBH was used. The data described above would suggest that the antihypertensive effect of picolinic acids is
related to their inhibitory action of DBH. In our previous studies (7), the depletion of brain or heart NE by these picolinic acids resulted from in vivo inhibition of brain or heart DBH. In this paper we present evidence that the picolinic acid derivatives inhibit in vivo DBH in the blood vessels. This inhibition of DBH in blood vessels is considered to be one of the mechanisms of antihypertensive or hypotensive actions of these compounds.

MATERIALS AND METHODS

Male Wistar rats weighing approx. 150 g were kept in a constant temp. and sound environment. The animals were exposed for at least ten days to regular light-dark cycles before initiation of the experiments. Rats were sacrificed by decapitation; the tissues were quickly removed. Tissues were weighed and stored at -80°C until biochemical analysis. FA, Br2FA and YP-279 (75 mg/kg or 25 mg/kg) were suspended in 0.5 ml of 0.5% carboxymethylcellulose (CMC) and injected i.p. into rats. Doses of the drugs used in most experiments were usually 75 mg/kg because the LDO is 75 mg/kg. Control rats were given an i.p. injection of 0.5 ml of 0.5% CMC. Statistical significance was determined by use of Student's t-test.

Radioisotopic study

14C-DA (57.3 mCi/mnmole) and 3H-NE (5–10 Ci/mnmole) were purchased from New England Nuclear. One fourth ml of diluted radioisotope solution (5 μCi): 20 μg of DA, 0.17 μg of NE, was administered into the tail vein. Rats were pretreated with CMC, FA, Br2FA or YP-279 0.5, 1, 3 or 6 hr before the injection of labelled catecholamines. Animals were sacrificed 60 min (14C-DA injection) and 15 min (3H-NE injection) following the administration of the labelled compounds. Tissues were homogenized in 7.5 ml of 0.4 N perchloric acid solution containing 50 μg of DA, NE, normetanephrine (NM), homovanillic acid (HVA) and 3-methoxytyramine (3-MT) with a glass tissue homogenizer. Ethylenediamine tetraacetic acid (10 mg) and Na2S2O3 (5 mg) were added as stabilizers, before homogenization. After the first homogenization and centrifugation, the precipitates were resuspended in 7.5 ml of 0.4 N perchloric acid, homogenized and centrifuged and the supernatants were saved. These two fractions (the first and the second supernatant) were combined and neutralized to pH 5.0 by addition of 5 N Na2CO3. The tissue extracts were passed over an 80 x 5 mm Amberlite CG-120 (Na+) column. The amines in the extracts were adsorbed to the resin and were thus separated from the acid and neutral catabolites which passed through in the effluent. The method of Carlson and Waldeck (6) was used, employing a modified elution procedure (7). Eluting with 50 ml of 0.4 N HCl, both acids and neutral catabolites were found in this fraction. The adsorbed amines was eluted with 50 ml of 1 N HCl followed by 20 ml of 2 N HCl. Each fraction containing 5 ml was collected. NE was collected in fractions 5 to 7 and DA was collected in fractions 11 to 13 (each fraction representing 5 ml). Further characterization of acid and neutral catabolites was not performed. One fourth ml of each fraction was transferred to scintillation vials, evaporated and 10 ml of scintillation fluid, Bray's solution (120 g naphthalene, 0.4 g POPOP, 8 g PPO, 200 ml methanol, 40 ml ethylene glycol, 21 dioxane) was added. Radioactivity was de-
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DBH assay: Rat tissues were homogenized with glass homogenizers in 10 volumes of cold tris (hydroxymethyl) aminomethane (Tris) buffer, 0.005 M, pH 7.4 containing Triton X-100, 0.1%. Homogenates were centrifuged at 15,000 x g for 10 min and portions of the supernatants were used for assay of DBH. DBH activity was not detectable in the pellets. DBH activity was measured by the method of Molinoff et al (8). The two stage assay for DBH is based on the conversion of tyramine to octopamine by DBH, followed by N-methylation of the β-hydroxylated product with a 14C-labelled methyl group from S-adenosylmethionine. The second reaction is catalyzed by bovine adrenal phenylethanolamine N-methyltransferase (PNMT), partially purified by the method of Axelrod (9) through the ammonium sulfate precipitation and then passed through Sephadex G-200 to separate PNMT from DBH. The reaction mixture contained: 200 μl of tissue preparation, corresponding to 1 to 10 mg of wet tissue wt; 0.12 μmole of ascorbic acid pH 6.0; 0.3 μmole of tyramine pH 6.0; and 10 μmoles of Tris buffer, pH 6.0. Sufficient catalase (1500 units) and CuSO4 (3 to 10 μmoles for hearts, 1 to 5 μmoles for aorta, 0.3 to 1 μmole for renal artery and 0.3 to 1 μmole for mesenteric artery) were added to give maximal activity. The total volume of the first stage of the assay was 310 μl. Tissue homogenates heated at 90–100°C for 5 min were used as blanks. The blanks for all tissues used were usually 300±50 cpm (n=15). DBH activity per mg tissue was calculated from the curve plotting DBH activity as a function of amounts of tissue added (mg wet wt.). To obtain the maximal activity of DBH in individual amounts of each tissue, different concentrations of CuSO4 indicated above were added to each reaction mixture. DBH activity obtained by the method as described above was linear as a function of amounts of tissue added. One unit of DBH activity was defined as 1 pmole synephrine formed per min per mg tissue.

Norepinephrine determination: Norepinephrine was isolated by alumina adsorption (10) and estimated fluorometrically after ferricyanide oxidation of 0.4 ml of the final acid eluate. Norepinephrine standard was added to aliquots of tissue and the recovery was estimated. All values were corrected for recoveries of 70±10% S.D. Blood vessels were carefully cleaned of adhering tissue with forceps and a small nylon brush as described by Koletsky et al (11), frozen on dry ice and stored at −80°C prior to analysis. Seven aortas or superior mesenteric arteries were pooled for each experiment. Four to six separated experiments were performed.

Concentration of FA, Br2FA and YP-279 in rat blood

The levels of the drugs in rat tissues were estimated by measuring their inhibitory effect on purified bovine adrenal dopamine β-hydroxylase. Bovine adrenal dopamine β-hydroxylase was highly purified by charcoal (acid-washed norite) treatment, ammonium sulfate fractionation, calcium phosphate gel treatment, ethanol fractionation and DEAE
cellulose column chromatography according to Friedman and Kaufman (12). The preparation of the dopamine \( \beta \)-hydroxylase used in these experiments did not contain detectable endogenous inhibitors. This was demonstrated by mixing the enzyme preparation of each step with an aliquot of the preparation of the succeeding step. The activity of this mixture was compared with the calculated sum of the individual activities (7). Inhibitory substances in the blood of rats which were pretreated with 75 mg/kg of FA, Br\(_2\)FA and YP-279 or 0.5 ml of 0.5% CMC were extracted from the blood with 5 volumes of absolute ethanol. The recovery of the picolinic acids added to rat blood by the ethanol extraction procedure was consistently above 90%. Appropriate volumes (10–1000 \( \mu l \)) of the ethanol extract which inhibited DBH by 30–70% were transferred to test tubes and evaporated. Inhibitory activity of this extract on purified DBH was determined by measuring DBH activity spectrophotometrically according to Creveling et al (13). The amounts of the compound were made as follows: 500 \( \mu l \) of ethanol extracts from the rat blood which contained 0–5000 ng/ml of these compounds was added to test tubes and evaporated. To each tube, an incubation mixture containing purified DBH was added and the inhibitory activity determined. Significant amounts of endogenous inhibitors were not found in ethanol extracts from the blood of rats treated with 0.5% CMC alone.

Measurement of blood pressure

The hypertensive rats were adult males derived from the colony of SHR produced by selected inbreeding of a strain of Wistar rats (14). Systolic blood pressure in the tail of SHR or normotensive Wistar rats was measured plethysmographically in unanaesthetized rats (15). The ability of FA to lower blood pressure was determined graphically by the method of Litchfield and Wilcoxon (16). The dose of drug which produces a lowering of blood pressure of 20 mmHg in 50% of the population was defined as the ED\(_{50}\). Blood pressure was measured at 1, 3 and 6 hr after various doses of FA (0.5–75 mg/kg) using 6 to 8 normotensive or hypertensive rats per group.

RESULTS

Time course studies on the reduced synthesis of \( ^{14} \text{C-NE} \) by pretreatment with FA, Br\(_2\)FA and YP-279 in blood vessels of the rat

In vivo inhibition of DBH by FA, Br\(_2\)FA and YP-279 in blood vessels was studied by following the time course of the reduction of \( ^{14} \text{C-NE} \) biosynthesis at various times after a 75 mg/kg dose of each compound (Table 1). Five \( \mu \)Ci of \( ^{14} \text{C-DA} \) were injected into rats i.v. 60 min before sacrifice. The biosynthesis of NE decreased rapidly and maximum inhibition of NE biosynthesis was attained at 0.5–1 hr in all tissues examined. This inhibition coincided approx. with peak blood concentrations of the drugs (Fig. 1). The biosynthesis of NE in the blood vessels approached control levels at 6 hr. At 6 hr, NE biosynthesis in the heart and adrenal glands was still considerably below the control levels.

Effect of FA, Br\(_2\)FA and YP-279 on the uptake of \( ^{3} \text{H-NE} \) into rat heart

Rats pretreated with FA, Br\(_2\)FA or YP-279, 75 mg/kg were given an i.v. injection of
TABLE 1. Effect of FA, Br₂FA and YP-279 on Norepinephrine biosynthesis

| Drug     | Time post drug (hr) | Aorta | Mesenteric artery | Renal artery | Carotid artery | Heart | Adrenal gland |
|----------|---------------------|-------|------------------|--------------|----------------|-------|---------------|
|          |                     |       |                  |              |                |       |               |
| Control  | 0.60 ± 0.08         | 0.91 ± 0.10 | 1.20 ± 0.15 | 0.21 ± 0.02 | 0.56 ± 0.02 | 0.30 ± 0.02 |
| (CMC)    |                     |       |                  |              |                |       |               |
| FA       | 0.5 0.03*           | 0.13 ± 0.02* | 0.11 ± 0.03* | 0.02*        | 0.13 ± 0.02* | 0.11 ± 0.01* |
| (75 mg/kg) | 1 0.17 ± 0.02* | 0.28 ± 0.02* | 0.40 ± 0.09* | 0.03* ± 0.01 | 0.11 ± 0.02* | 0.11 ± 0.01* |
|          | 3 0.39 ± 0.03* | 0.36 ± 0.05* | 0.70 ± 0.10 |              | 0.17 ± 0.01* | 0.13 ± 0.01* |
|          | 6 0.59 ± 0.07 | 0.58 ± 0.08* | 1.10 ± 0.12 |              | 0.22 ± 0.03* | 0.14 ± 0.03* |
| Br₂FA    | 0.5 0.05 ± 0.01* | 0.22 ± 0.03* | 0.02*        | 0.02*        | 0.08 ± 0.01* | 0.06 ± 0.03* |
| (75 mg/kg) | 1 0.14 ± 0.02* | 0.32 ± 0.02* | 0.13 ± 0.03* | 0.02*        | 0.08 ± 0.01* | 0.06 ± 0.01* |
|          | 3 0.31 ± 0.04* | 0.64 ± 0.08 | 0.30 ± 0.08* |              | 0.17 ± 0.03* | 0.20 ± 0.02* |
|          | 6 0.51 ± 0.06 | 0.90 ± 0.08 | 1.10 ± 0.04 |              | 0.35 ± 0.03* | 0.32 ± 0.03 |
| YP-279   | 0.5 0.44 ± 0.04* | 0.44 ± 0.05* | 0.32 ± 0.06* | 0.11* ± 0.02 | 0.21 ± 0.02* | 0.11 ± 0.01* |
| (75 mg/kg) | 1 0.20 ± 0.02* | 0.25 ± 0.01* | 0.31 ± 0.05* | 0.05 ± 0.01 | 0.13 ± 0.02* | 0.04 ± 0.01* |
|          | 3 0.36 ± 0.04* | 0.44 ± 0.08* | 0.42 ± 0.03* |              | 0.18 ± 0.01* | 0.06 ± 0.01* |
|          | 6 0.35 ± 0.05 | 0.70 ± 0.09 | 0.90 ± 0.12 |              | 0.28 ± 0.02* | 0.13 ± 0.02* |

Rat were pretreated with FA, Br₂FA and YP-279 at various times (0.5–6 hr) before administration of ¹⁴C-DA. Five μCi of ¹⁴C-DA were injected 60 min before sacrifice. Control rats were pretreated with 0.5% CMC. Results from 9 hearts were averaged and three groups of pooled vessels (three animals per group) and 9 groups of a pair of adrenal glands were averaged. The results are expressed as the mean ± S.E.

* Significantly different from control (P<0.05)

5 μCi ³H-NE 60 min after the drugs and were sacrificed 15 min later. Control rats were injected with 0.5% CMC. The uptake of ³H-NE into the heart was not affected by the pretreatment with FA, Br₂FA and YP-279 (Table 2). These three compounds were found to have no effect on the release of NE from mouse heart (7).
TABLE 2. Effect of picolinic acid derivatives on the uptake and catabolism of \(^3\)H-NE in rat heart.

| Drug   | Dose (mg/kg) | Total (pmoles/g heart) | NE (pmoles/g heart) | NM (pmoles/g heart) | Acid and Neutral (pmoles/g heart) |
|--------|--------------|------------------------|---------------------|---------------------|-----------------------------------|
| Control |              | 12.9 ± 0.67            | 9.18 ± 0.36         | 1.10 ± 0.17         | 1.90 ± 0.26                      |
| FA     | 37.5         | 14.0 ± 0.33            | 9.98 ± 0.97         | 1.21 ± 0.21         | 2.21 ± 0.29                      |
| Br\(_2\)FA | 75        | 12.0 ± 0.54            | 9.03 ± 0.37         | 1.10 ± 0.18         | 2.01 ± 0.32                      |
| YP-279 | 75           | 12.4 ± 0.36            | 8.86 ± 0.20         | 1.10 ± 0.17         | 2.10 ± 0.33                      |

Five μCi of \(^3\)H-NE were injected i.v. 15 min before sacrifice. Rats were pre-treated with 75 mg/kg of picolinic acid derivatives (i.p.) 1 hr before \(^3\)H-NE injection. Results from 5 hearts were averaged.

TABLE 3. Effect of FA, Br\(_2\)FA and YP-279 on NE contents in blood vessels

Results are the mean ± standard error and are corrected for recovery loss. Numbers in parentheses indicate the number of experiments. Seven aortas or 7 superior mesenteric arteries were pooled for each determination.

* Significantly different from control (p< .05)
** Significantly different from control (p< .01)

Effect of FA, Br\(_2\)FA and YP-279 on NE levels of blood vessels

In Table 3, the effects of DBH inhibitors on aorta and mesenteric artery NE concentration in the rat are compared during the first 4 hr after administration of 37.5 and 75 mg/kg of FA, Br\(_2\)FA or YP-279. Both aortic and mesenteric artery NE contents were significantly reduced at 4 hr after FA or Br\(_2\)FA (Table 3). However, YP-279 reduced only mesenteric artery NE content significantly at 4 hr after the administration.

Effect of FA, Br\(_2\)FA or YP-279 on DBH activity in rat heart and blood vessels

DBH activity in rat heart and some blood vessels is shown in Table 4. Mesenteric
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TABLE 4. Effect of FA, Br₂FA and YP-279 on DBH activity

| Tissue     | Control CMC | FA (mg/kg) 25 | 50 | Br₂FA (mg/kg) 25 | 50 | YP-279 (mg/kg) 50 | 75 |
|------------|-------------|---------------|----|-----------------|----|-----------------|----|
| Heart      | 1.05 ± 0.31 | 0.29** (3)    |    | 0.20** (3)      |    | 0.14*** (3)     |    |
|            |             | 0.14*** (3)   |    | 0.10*** (4)     |    | 0.35** (4)      |    |
|            |             | 0.22** (4)    |    | 0.35** (4)      |    | 0.35** (4)      |    |
| Aorta      | 0.21 ± 0.09 | 0.21 (3)      |    | 0.19 (3)        |    | 0.12** (3)      |    |
|            |             | 0.13** (3)    |    | 0.11** (3)      |    | 0.13** (3)      |    |
|            |             | 0.07** (3)    |    | 0.07** (3)      |    | 0.05** (3)      |    |
| Mesenteric | 2.90 ± 0.10 | 1.90 ± 0.10   |    | 1.41** (3)      |    | 0.98** (3)      |    |
| Artery     | 1.047 ± 0.06| 0.35 ± 0.06   |    | 0.25 ± 0.06     |    | 0.33 ± 0.06     |    |
|            |             | 0.30 ± 0.06   |    | 0.30 ± 0.06     |    | 0.30 ± 0.06     |    |
| Renal      | 1.92 ± 0.03 | 0.30 ± 0.03   |    | 0.25 ± 0.03     |    | 0.30 ± 0.03     |    |
| Artery     | 0.66** (3)  | 0.10 ± 0.04   |    | 0.04 ± 0.04     |    | 0.03 ± 0.04     |    |

Dopamine β-hydroxylase activity (unit *)

* unit: 1 pmole synephrine formed/min/mg tissue (wet wt.)
** Significantly different from control (P<0.05)
*** Significantly different from control (P<0.01)

Drugs were administered i.p. and rats were sacrificed 1 hr after. Numbers in parentheses indicate the number of experiments. Results are the mean ± standard error.

artery contained the highest activity among tissues tested. Dose-response curves were determined for the effect of FA, Br₂FA or YP-279 on DBH activity in various rat tissues (Table 4). These drugs inhibited effectively DBH activity in the blood vessels and heart.

Concentrations of FA, Br₂FA and YP-279 in rat blood

Rats injected with 75 mg/kg of FA, Br₂FA or YP-279 were sacrificed 0.5, 1, 3 and 6 hr later. Control rats were injected with CMC. Inhibitory activities of ethanol extracts of the blood samples to DBH were determined and the concentration of each drug was calcu-

![Fig. 2](image-url)  
**Fig. 2.** Standard inhibition curve of FA, Br₂FA and YP-279 to purified DBH. One ml of rat blood which had contained 0-5000 ng of FA, Br₂FA or YP-279 was mixed with 5 ml of absolute ethanol and denatured proteins were removed by centrifugation. 0.5 ml of this supernatant was transferred to test tubes and evaporated. Inhibition of purified DBH by the residues was determined.
lated using standard inhibition curves of the drugs to purified bovine DBH. Standard inhibition curves were obtained from per cent inhibition of highly purified DBH by ethanol extracts of rat blood to which each compound (0-5000 ng/ml) had been added (Fig. 2). Ethanol extracts of control rat blood did not inhibit purified DBH if less than 1 ml of this extract was added. As indicated in Fig. 1, the concentrations of FA, Br₂FA and YP-279 in the blood increased rapidly in the first 30 min.

**Effect of FA, Br₂FA and YP-279 on blood pressure of normotensive Wistar rats and SHR**

Although an i.p. injection above 25 mg/kg lowered blood pressure significantly in normotensive rats (7), i.p. administration of lower doses (FA 3.0 mg/kg, Br₂FA 3.0 mg/kg and YP-279 8.0 mg/kg) of these drugs to SHR lowered the blood pressure effectively (Fig. 3). As shown in Table 5, the dose of FA sufficient to lower blood pressure by 20 mmHg in 50% of the population (ED50) of SHR was about one tenth of the dose required for normotensive rats. The route of administration did not affect the blood pressure lowering action of this drug.

![Graph showing effect of FA, Br₂FA and YP-279 on blood pressure](image)

**Fig. 3.** Effect of FA, Br₂FA and YP-279 on the systolic blood pressure in SHR. Experimental animals were given 3.0 mg/kg (FA and Br₂FA) and 8.0 mg/kg (YP-279). Vertical bars indicate the standard error obtained from eight determinations.

Statistically significant at p<0.05 when compared to control values.

**Table 5. ED50 of FA (hypotensive effect)**

| Animal   | Route | ED50 (mg/kg) (mean ± S.E.) |
|----------|-------|-----------------------------|
| Normotensive | I.P.  | 25 ± 5                      |
|           | P.O.  | 25 ± 7                      |
| SHR      | I.P.  | 3.1 ± 1.0                   |
|           | P.O.  | 3.2 ± 1.2                   |

Blood pressure was measured 1, 3, 6 hr after FA and 20% depression of blood pressure in 50% of the population was judged to be effective. Various doses of FA (0.5-75 mg/kg) were administered i.p. or p.o. to 6 to 8 rats (normotensive or SHR) per group.
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DISCUSSION

FA, Br₂FA and YP-279 are potent inhibitors of dopamine β-hydroxylase in vitro (1). Although evidence has accumulated that these compounds deplete heart or brain catecholamines, there was no direct evidence that catecholamines in blood vessels were reduced due to in vivo DBH inhibition by these inhibitors. The present studies show that FA, Br₂FA and YP-279 inhibit the conversion of DA to NE in vivo in the blood vessels. Evidence for the inhibitory activity of these drugs in vivo was 1) the specific reduction of tissue NE concentrations in rats (2, 17) and 2) the drug-induced changes in the conversion of DA to NE in several tissues of the rat consistent with plasma levels of the drugs.

The reason why inhibition of NE biosynthesis in the heart and adrenal glands did not return to the control levels at 6 hr after these drugs is attributed to higher concentrations of the drugs in the heart and adrenal glands (7). Mechanism of the retention of the drugs in these tissues is not clear.

The inhibition of the biosynthesis of heart NE from DA by FA, Br₂FA and YP-279 resulted in the consistent elevation of acid and neutral catabolites and only slight increase in dopamine (7). This indicated that monoamine oxidase plays a more important role than DBH in the catabolism of exogenously administered dopamine. FA does not inhibit monoamine oxidase in vivo or in vitro (18). Br₂FA and YP-279 also do not inhibit rat brain and liver monoamine oxidase in vitro up to 10⁻³ M (unpublished observation). The present studies as well as previous ones (7) show that FA, Br₂FA and YP-279 are all effective hypotensive agents in normotensive Wistar rats and also effective antihypertensive drugs in SHR. The present studies also show that the effect of FA on blood pressure in both SHR and normotensive rats is similar by either the p.o. or i.p. route (Table 5). It is possible that the hypotensive or antihypertensive effect of these drugs is partially related to the in vivo inhibition of DBH and their ability to reduce the biosynthesis of NE. It also may be considered that increase in dopamine contents in blood vessels after these drugs might affect the function of blood vessels. However, the increase in dopamine levels of blood vessels after drugs could not be demonstrated because of the extremely low content in the blood vessels. Accordingly, contribution of dopamine mechanism to antihypertensive activities of these drugs has not been clarified. In a study of several 5-alkylpicolinic acids, Suda et al. (5) reported that a reduction of blood pressure was more marked when a more potent inhibitor of DBH was used. Moreover, Korduba et al. (19) have recently reported that after administration of 5-(n-butyl) picolinamide (Sch 10595), which is an effective inhibitor of DBH in vivo, the blood pressure reduction correlated with the reduction of endogenous NE levels in sympathetically innervated tissues. The antihypertensive effect of these drugs in SHR has already been reported by Hidaka and Takeya (2). The present studies and our previous studies (7) show that these picolinic acid derivatives effectively reduce the systolic blood pressure in SHR at far lower doses (one-tenth) than in normotensive rats. However, with the present data, a direct relationship between the inhibition of DBH and the antihypertensive effect of these drugs cannot be proven as an evaluation of the in vivo inhibition of DBH in the SHR using ¹⁴C-DA as precursor was not carried out. More-
over, blood pressure decreases immediately (within a few min) after injection of these drugs (20) and this rapid decrease in blood pressure cannot be explained by the relatively slow decrease in endogenous NE levels after these drugs. There are many drugs, such as α-methyltyrosine, reserpine and guanethidine, etc. which affect adrenergic function and exhibit anti-hypertensive action. From the standpoints of reduced contents of tissue NE after drugs affecting adrenergic function mentioned above, picolinic acid and its derivatives appear to be similar to these drugs, but the antihypertensive mechanism of picolinic acid derivatives seem to be different from other drugs. For example, α-methyltyrosine reduces not only NE but also DA and epinephrine. Reserpine also reduces all catecholamines in the tissues as well as other biogenic amines and guanethidine suppresses the response mediated by both α- and β-adrenergic receptors. In addition, picolinic acid derivatives do not affect the response mediated by α- or β-adrenergic receptors but do affect the responses mediated by other unknown mechanisms (21, 22). Although we do not exclude the possibility that the antihypertensive action or hypotensive action of these compounds would be due to unknown actions of these drugs rather than to their DBH inhibitor activities, it might be possible that the antihypertensive or hypotensive effect of these picolinic acid derivatives is partly due to their DBH inhibitory actions. The fact that these drugs inhibit the conversion of DA to NE in the blood vessels and also have an antihypertensive effect suggests the important role of catecholamines in the occurrence of hypertension.

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