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RELAXATION OF ISOLATED AORTA OF THE RABBIT BY PICOLINIC ACIDS

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1 When the isolated thoracic aorta of the rabbit was contracted with prostaglandin $F_{2\alpha}$, 5-alkylpicolinic acids produced dose-dependent relaxations.

2 Picolinic acid, 2,5-pyridinedicarboxylic acid and 5-acetylpicolinic acid which do not have the 5-alkyl residue failed to relax blood vessels.

3 The vascular relaxation was dependent on the number of carbon atoms in the 5-alkyl compounds.

4 Relaxations which occurred with 5-alkylpicolinic acids were not affected by pretreatment with either propranolol or atropine.

5 It is concluded that the 5-alkyl residue is necessary for the vascular relaxation with 5-alkylpicolinic acid and that it was not produced by stimulation of β-adrenoceptors or cholinoceptors but rather through an activation of the basic process.

Introduction

Fusaric (5-butylpicolinic) acid (FA) is a potent inhibitor of a purified bovine adrenal dopamine β-hydroxylase [EC: 1,14,2,1] (DBH) (Hidaka, Nagatsu, Takeya, Takeuchi, Suda, Kojiri, Matsuzaki & Umezawa, 1969) and an effective antihypertensive and hypotensive agent in man (Terasawa & Kameyama, 1971) and other mammals (Hidaka & Takeya, 1972; Hidaka, Nagasaka & Takeda, 1973; Hidaka, Hara, Harada, Hashizume & Yano, 1974a). A significant reduction in blood pressure was demonstrated in both spontaneously hypertensive rats (SHR) and normotensive rabbits after intravenous and intraperitoneal injections or oral administration of fusaric acid or 5-(3′,4′-dibromobutyl)picolinic acid (dibromorfusarate Br_2FA) (Hidaka & Takeya, 1972; Hidaka, Hara, Harada & Asano, 1974b).

Thus, it is conceivable that the reduction in blood pressure with FA is the result of reduced formation of tissue noradrenaline content through inhibition of DBH (Hidaka, Shoka, Hashizume, Takemoto & Yamamoto, 1975). On the other hand, decrease in blood pressure with FA is observed immediately (1–2 min) after intraveneous injection (Hidaka *et al.*, 1974b). Such a rapid hypotensive effect of FA cannot be explained only by decrease in tissue noradrenaline through inhibition of DBH. We also found that FA does not release tissue catecholamines (Hidaka *et al.*, 1974a). Therefore, the possibility that FA lowers the blood pressure through mechanisms other than inhibition of DBH or release of catecholamines must be considered. Recently, we demonstrated that FA causes a relaxation of rabbit arterial strips contracted with either prostaglandin $F_{2\alpha}$ or KCl and also inhibits the contraction induced by various agonists (Asano & Hidaka, 1975; Hidaka & Asano, 1976).

The vascular relaxation produced by various derivatives of picolinic acid and pyridine has been studied. The structure-activity relationship between the derivatives of FA was demonstrated by using several analogues of FA such as pyridine-monocarboxylic acid, pyridine-dicarboxylic acids, 5-acetylpicolinic acid and 5-alkylpicolinic acids.

Methods

Albino rabbits of either sex weighing 1.9–2.5 kg were killed by a blow on the head and exsanguinated. The thoracic aorta (2.5–5.0 mm outside diameter) was quickly excised and after removal of adventitial connective tissue, the aorta was cut to make a spiral strip, 2–3 mm in width and 25–35 mm in length (Lewis & Koessler, 1927; Furchgott & Bhadrakom, 1953). The spiral strip was fixed vertically between hooks in a water-jacketed tissue bath containing 40 ml of modified Ringer Locke solution having the following composition (mM): NaCl 140.9, KCl 5.6, $CaCl_2 2H_2O$ 2.2, $NaHCO_3$ 14.9 and dextrose 5.6. Solutions were maintained at 37 ± 0.5°C and bubbled with a mixture of 95% O_2 and 5% CO_2. The upper end of the strip

was connected to the lever of a force-displacement transducer (SB-1T, Nihon Kohden Kogyo Co., Tokyo, Japan) by a silk thread. An initial resting tension of 1 g was applied to the aorta. Before the experiments were started, preparations were allowed to equilibrate for 1 h in the bathing solution. During this period, the solutions were replaced every 20 minutes.

Cumulative dose-response curves for vascular responses to the agonists were obtained by increasing the concentrations by a factor of about 3, while the previous dose remained in contact with the tissue (van Rossum, 1963). Each concentration was added only after the effects of the previous concentration had reached a maximum and remained constant. Maximum responses obtainable were assumed when a concentration of 3×10^{-3} M of each agonist was added. To compare agonists which relax blood vessels, papaverine in a concentration of 1×10^{-4} M was added at the end of each series of experiments and relaxation induced by papaverine was taken as 100% (Toda, 1974; Buckner & Saini, 1975). Stock solutions of drugs were added directly to the bathing solution in a volume of 0.4 ml to give the final concentrations desired. Only one cumulative dose-response curve to a compound was obtained from a single preparation. Usually paired strips from the same animal were given different treatments and, in drug antagonism studies, one strip was used to obtain the control dose-response curve. Effects of various blocking agents such as propranolol, atropine, aminophylline, haloperidol and ouabain on relaxations induced by picolinic acids were determined with these blocking agents before the addition of prostaglandin $F_{2\alpha}$. Whenever an ED_{50} value was determined, responses to the agonists were calculated as a percentage of the maximum relaxation obtained with that agonist. The ED_{50} value was obtained visually from a plot of percentage relaxation v log concentration of the agonist and expressed as the negative logarithm (–log molar ED_{50}). Results shown in the text, tables and figures are expressed as the mean value ± s.e. Comparison of the results was made by Student's t-test. Statistical significance was assumed when $P < 0.05$.

Drugs and chemicals

The following drugs were used: (±)-propranolol hydrochloride (Sigma), atropine sulphate (Katayama), aminophylline, haloperidol (Serenace, Speeling Dainippon Pharmaceutical Co., Ltd.), ouabain (Merck), prostaglandin $F_{2\alpha}$ (Prostarmon F, Ono Pharmaceutical Co., Ltd.), papaverine hydrochloride (Katayama), (–)-isoprenaline hydrochloride (Sigma), N^6,$O^{2'}$dibutyryl cyclic adenosine 3':5'-monophosphoric acid monosodium salt (Boehringer), picolinic acid sodium salt (Tokyo Kasei Kogyo Co., Ltd.), nicotinic acid sodium salt (Tokyo Kasei Kogyo Co., Ltd.), isonicotinic acid (Wako), 2-aminopyridine (Wako), 2,3-pyridinedicarboxylic acid (Wako) and 2,5-pyridinedicarboxylic acid (Wako). 2,4-Pyridinedicarboxylic acid, 2,6-pyridinedicarboxylic acid, 5-acetylpicolinic acid, 5-methylpicolinic acid, 5-butylpicolinic acid (FA), 5-pentylpicolinic acid, 5-(monobromomethyl)picolinic acid, 5-(3',4'-dibromobutyl)picolinic acid (Br_2FA), 5-(*N*,*N*-dimethylthiocarbomoylthiomethyl)picolinic acid (YP-279) and 5-(*N*,*N*-dimethylthiocarbamoylthiomethyl)picolinic acid monomethylamide (YP-614) were synthesized by Dr I. Matsumoto of Banyu Pharmaceutical Co., Ltd. Picolinic acids tested are shown in Table 1.

Picolinic acid derivatives were prepared in distilled water and pH was adjusted to 7.4 with sodium hydroxide. Other drugs (with the exception of papaverine, which was prepared in 0.9% w/v NaCl solution (saline)) were prepared daily in Ringer Locke solution and kept on ice during the course of the experiment.

Results

Relaxation induced by picolinic acid derivatives in aortic strips

The addition of prostaglandin $F_{2\alpha}$ in a concentration of 5×10^{-7}M caused a sustained contraction in aortic strips (Figure 1a). When preparations were contracted with 5×10^{-7} M prostaglandin $F_{2\alpha}$, different degrees of tension (0.1–2.4 g) were obtained. The mean value of the tension developed in thoracic aortae was 991 ± 97 mg ($n = 99$). Preparations thus contracted showed good relaxation in response to vasodilators. The addition of 5-pentylpicolinic acid in concentrations ranging from 3×10^{-6} M to 3×10^{-3} M elicited a dose-dependent relaxation (Figure 1b). The slope of the dose-response curve for relaxation to 5-pentylpicolinic acid remained the same provided that the initial tension developed by the prostaglandin $F_{2\alpha}$ lay between 0.5 and 2.4 grams. The slope of the curve was reduced when the initial tension was less than 0.4 gram. In another experiment, the relationship between the concentration of prostaglandin $F_{2\alpha}$ and the 5-pentylpicolinic acid-induced relaxation was examined. When the preparation was contracted with doses of prostaglandin $F_{2\alpha}$ (5×10^{-7} M, 1×10^{-6} M and 2×10^{-6} M) the maximum relaxation obtained with 5-pentylpicolinic acid was the same; but concentrations of 5×10^{-8} M and 5×10^{-6} M produced different maximal effects. Consequently, prostaglandin $F_{2\alpha}$ (5×10^{-7}M) was usually used to produce a tension of approximately 1.0 gram. If this concentration produced a tension of less than 1.0 g, it was raised to 1×10^{-6} M and in a few cases to 2×10^{-6} M until a tension within the range 1.0–2.0 g was produced.

The additions of 5-methylpicolinic acid, 5-butylpicolinic acid (FA), 5-pentylpicolinic acid, 5-(bromomethyl)picolinic acid, 5-(3',4'-dibromobutyl-picolinic acid (Br_2FA), 5-(*N*,*N*-dimethylthiocarbamoylthio-

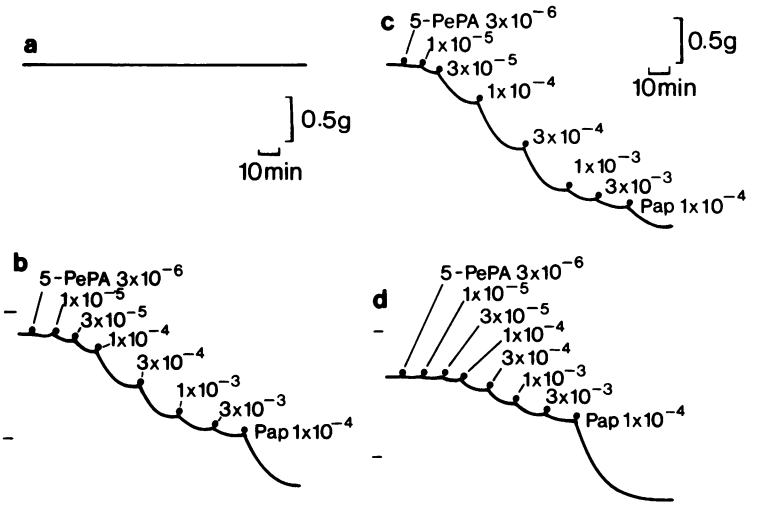


Figure 1 Responses of a strip of rabbit thoracic aorta to 5-pentylpicolinic acid. The preparations were contracted with prostaglandin $F_{2\alpha}$ 5×10^{-7} M. Horizontal line on left of each tracing indicates the level of tension before the addition of prostaglandin $F_{2\alpha}$. (a) Control without 5-pentylpicolinic acid showing that the contraction of the preparation by prostaglandin is sustained for at least 2 hours. (b) The 5-pentylpicolinic acid (5-PePA) induced dose-dependent relaxation of the preparation contracted with prostaglandin $F_{2\alpha}$. Further relaxation induced by papaverine 1×10^{-4} M (Pap). (c) Relaxation induced by 5-pentylpicolinic acid was enhanced by pretreatment with haloperidol and in (d) was attenuated by ouabain. Haloperidol and ouabain were preincubated with the strips for 10 min before the addition of prostaglandin $F_{2\alpha}$. Molar concentrations are shown.

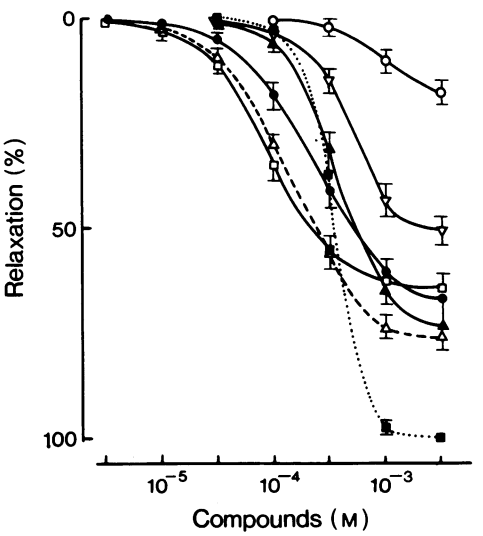


Figure 2 Dose-relaxation curves for 5-alkylpicolinic acids in strips of thoracic aorta. Tension induced by prostaglandin $F_{2\alpha}$ (5×10^{-7} M) was 1025×77 mg ($n = 19$) and papaverine (1×10^{-4} M)-induced maximum relaxation was 1461 ± 114 mg ($n = 19$). The relaxation induced by 1×10^{-4} M of papaverine was taken as 100%. Vertical bars represent s.e. Figures in parentheses indicate the number of preparations used. (○) 5-Methylpicolinic acid; (●) 5-butylpicolinic acid; (□) 5-pentylpicolinic acid; (■) 5-(bromomethyl)picolinic acid; (△) 5-(3′,4′-dibromobutyl)picolinic acid; (▲) 5-(*N,N*-dimethylthiocarbamoylthiomethyl)picolinic acid; ( ) 5-(*N,N*-dimethylthiocamoylthiomethyl)picolinic acid monoethylamide.

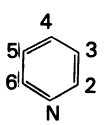
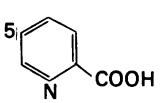
methyl)picolinic acid (YP-279) and 5-(*N,N*-dimethylthiocarbamoylthiomethyl)picolinic acid monomethylamide (YP-614) caused dose-dependent relaxations (Figure 2). However, pyridinemonocarboxylic acids (such as picolinic acid, nicotinic acid and isonicotinic acid), pyridinedicarboxylic acids (such as 2,3-, 2,4-, 2,5-, and 2,6-pyridinedicarboxylic acid) and other compounds (such as 2-aminopyridine, 5-acetylpicolinic acid) failed to relax the aorta. The average percentage relaxation produced by picolinic acid derivatives (1 mM) and the negative logarithm of the molar concentrations for the ED_{50} (−log molar ED_{50})

are shown in Table 1. Thus the 5-alkyl residue was necessary for the vascular relaxation produced by 5-alkylpicolinic acids and the 2-carboxyl groups also contributed. According to the ED_{50} values obtained, the potency of the vascular relaxing response of 5-alkylpicolinic acids was in the order of 5-pentylpicolinic acid > Br_2FA > FA > YP-279 > 5-(bromomethyl) picolinic acid > YP-614 > 5-methylpicolinic acid.

Effects of propranolol, atropine, aminophylline, haloperidol and ouabain on 5-alkylpicolinic acids-induced relaxations

The relaxation of the aortic strip produced by 5-alkylpicolinic acids was not affected by pretreatment with propranolol 5×10^{-6} M, atropine 1×10^{-5} M or by aminophylline 3×10^{-4} M (an adenosine antagonist) (Table 2). However dose-relaxation curves for FA, 5-pentylpicolinic acid, Br_2FA, YP-279, YP-614 and 5-methylpicolinic acid shifted to the left after pretreatment with 1×10^{-5} M haloperidol (Figure 1c and Table 3). When the preparations were pretreated with ouabain 2×10^{-7} M or 1×10^{-6} M, dose-relaxation curves for 5-alkylpicolinic acids were inhibited dose-dependently (Figure 1d and Table 3). Thus the relaxation caused by 5-alkylpicolinic acids was enhanced by haloperidol and inhibited by ouabain, while the ED_{50} values were not affected (Table 3). Relaxation of the thoracic aorta by isoprenaline, N^6,$O^{2'}$dibutyryl cyclic adenosine 3':5'-monophosphoric acid (dibutyryl cyclic AMP) or by aminophylline, was also enhanced by haloperidol and attenuated by ouabain (Table 3). Papaverine

Table 1 Arterial strip relaxation induced by picolinic acid and its derivatives

Compounds	*Pyridine* 	n	*% Relaxation**	*−Log molar ED_{50}*†
Picolinic acid	2: COOH	10	3.1 ± 1.1	
Nicotinic acid	3: COOH	9	3.6 ± 1.4	
Isonicotinic acid	4: COOH	8	1.4 ± 0.6	
2-Aminopyridine	2: NH_2	10	0.3 ± 0.2	
2,3-Pyridinedicarboxylic acid	2,3: COOH	8	5.0 ± 2.1	
2,4-Pyridinedicarboxylic acid	2,4: COOH	8	2.0 ± 1.0	
2,5-Pyridinedicarboxylic acid	2,5: COOH	9	3.3 ± 0.9	
2,6-Pyridinedicarboxylic acid	2,6: COOH	8	4.0 ± 2.1	
	Picolinic acid 			
5-Acetylpicolinic acid	5: $COCH_3$	8	2.7 ± 1.0	
5-Methylpicolinic acid	5: CH_3	8	10.2 ± 2.4	
5-Butylpicolinic acid	5: $CH_2CH_2CH_2CH_3$	19	60.1 ± 2.6	3.67 + 0.04
5-Pentylpicolinic acid	5: $CH_2CH_2CH_2CH_2CH_3$	9	62.1 ± 3.4	4.07 + 0.04
5-(Monobromomethyl)picolinic acid	5: CH_2Br	19	97.0 ± 1.0	3.40 + 0.06
5-(3',4'-Dibromobutyl)picolinic acid	5: $CH_2CH_2CHBrCH_2Br$	13	73.2 ± 1.4	3.87 + 0.05
5-(*N,N*-dimethylthiocarbamoylthiomethyl)picolinic acid	5: $CH_2SCSN(CH_3)_2$	11	65.4 ± 2.6	3.43 + 0.03
5-(*N,N*-dimethylthiocarbamoylthiomethyl)picolinic acid monomethylamide	5: $CH_2SCSN(CH_3)_2$ 2: $CONHCH_3$	15	43.1 ± 3.8	3.33 + 0.01

* Relaxation produced by a concentration of 1 mM expressed as a percentage of the maximum response produced by papaverine (1×10^{-4} M) which was taken as 100%. Tension induced by prostaglandin $F_{2\alpha}$ (5×10^{-7}M) was 1039 ± 61 mg ($n = 67$).

† The ED_{50} value was obtained visually from a plot of percentage relaxation *v* log concentration of the compound and expressed as the negative logarithm (−log molar ED_{50}).

n indicates the number of preparations used. Data are expressed as mean ± s.e. mean.

(1×10^{-4}M)-induced maximum relaxation was not affected by pretreatment with haloperidol, ouabain, propranolol, atropine or aminophylline.

Discussion

All 5-alkylpicolinic acids tested produced relaxation of the rabbit aorta. 5-Methylpicolinic acid, FA- and 5-pentylpicolinic acid caused a dose-dependent relaxation of the thoracic aorta and the potency depended on the number of carbon atoms in the 5-alkyl residue. 5-Pentylpicolinic acid and FA produced a greater relaxation than did 5-methylpicoloinic acid. The addition of a large molecule to the 5-alkyl residue of 5-alkylpicolinic acid potentiated the response. For example 5-(monobromomethyl)picolinic acid and 5-(*N,N*-dimethylthiocarbamoylthiomethyl)picolinic acid (YP-279) induced a more potent relaxation of the aorta than did 5-methylpicolinic acid. 5-(3′,4′-Dibromobutyl)picolinic acid (Br_2FA) proved to be more potent than FA in inducing vascular relaxation. However, picolinic acid, 2,5-pyridinedicarboxylic acid and 5-acetylpicolinic acid which do not have the 5-alkyl residue failed to relax the blood vessels. From these observations, it is presumed that the 5-alkyl residue is necessary for vascular relaxation with 5-alkylpicolinic acid.

Suda, Takeuchi, Nagatsu, Matsuzaki, Matsumoto & Umezawa (1969) discussed the relationship between the inhibition of DBH by 5-alkylpicolinic acids and their hypotensive effects. These workers demonstrated that picolinic acid and all 5-alkylpicolinic acids inhibit bovine adrenal DBH and that all of them had hypotensive effects. The degree of DBH inhibition is dependent on the number of carbon atoms in the 5-alkyl group and 5-butyl and 5-pentyl compounds are more potent than the others. When the carbon atoms in the 5-alkyl residue are increased to 4 or 5, the hypotensive effect becomes stronger, thus 5-butyl- and 5-pentyl-picolinic acids were more potent than 5-propionyl-, 5-ethyl- and 5-methyl-picolinic acids. It is interesting that the hypotensive effects of picolinic acid derivatives appear to correlate with both their vasodilator actions and their DBH inhibitory activities.

In our previous studies (Asano & Hidaka, 1975; Hidaka & Asano, 1976), 5-butylpicolinic acid (FA), one of the 5-alkylpicolinic acids was observed to have a direct relaxant action on blood vessels. FA decreased the contractor responses elicited with noradrenaline, histamine, 5-hydroxytryptamine, acetylcholine, angiotensin II and KCl in helical strips of rabbit aorta (Ao) and superior mesenteric (Sm), carotid (Cd), renal (R) and femoral (Fe) arteries. When blood vessels were contracted with either prostaglandin $F_{2\alpha}$ or KCl, FA caused a relaxation of Ao, Sm, Cd, R and Fe arteries in a dose-dependent fashion. FA has an anti-hyper-

Table 2 Effects of propranolol, atropine and aminophylline on the vascular relaxation produced by picolinic acid derivatives

		Control		*Propranolol‡ (5×10^{-6} M)*		*Atropine‡ (1×10^{-5} M)*		*Aminophylline‡ (3×10^{-4} M)*	
		n		n		n		n	
% Relaxation*	FA	19	60.1 ± 2.6	8	61.3 ± 5.3	8	54.6 ± 4.9	8	55.4 ± 4.7
	5-pentyl PA	9	62.1 ± 3.4	8	65.7 ± 4.2	8	57.6 ± 4.7	8	51.3 ± 4.8
	BR_2FA	13	73.2 ± 1.4	8	78.6 ± 2.8	8	75.1 ± 3.5	8	69.6 ± 2.5
	YP-279	11	64.5 ± 2.6	8	63.0 ± 4.6	8	58.3 ± 5.6	8	56.0 ± 4.8
	YP-614	15	43.1 ± 3.8	8	53.2 ± 6.0	8	48.5 ± 2.9	8	42.5 ± 4.8
−Log molar ED_{50}†	FA	19	3.76 ± 0.04	8	3.67 ± 0.05	8	3.81 ± 0.06	8	3.85 ± 0.06
	5-pentyl PA	9	4.05 ± 0.04	8	4.25 ± 0.08	8	4.03 ± 0.05	8	3.98 ± 0.10
	Br_2FA	13	3.95 ± 0.05	8	3.95 ± 0.05	8	3.92 ± 0.04	8	3.86 ± 0.05
	YP-279	11	3.50 ± 0.03	8	3.61 ± 0.06	8	3.60 ± 0.05	8	3.51 ± 0.03
	YP-614	15	3.41 ± 0.01	8	3.43 ± 0.03	8	3.41 ± 0.02	8	3.44 ± 0.03

FA = 5-butylpicolinic acid; 5-pentyl PA = 5-pentylpicolinic acid; Br_2FA = 5-(3′,4′-dibromobutyl)picolinic acid; YP-279 = 5-(*N,N*-dimethylthiocarbamoylmethyl)picolinic acid; YP-614 = 5-(*N,N*-dimethylthiocarbamoylthiomethyl)picolinic acid monethylamide.

* Percentage relaxation produced by 1×10^{-3} M of each compound. (see Table 1 and Figure 1).

† The ED_{50} value was obtained visually from a plot of percentage relaxation *v.* log concentration of a compound and expressed as the negative logarithm (−log molar ED_{50}). The maximum relaxation induced by the compound was taken as 100%.

‡ Propranolol, atropine or aminophylline was added to the bath 10 min before prostaglandin $F_{2\alpha}$.

n indicates the number of preparations used. Data are expressed as mean ± s.e. mean.

tensive effect in hypotensive patients (Terasawa & Kameyama, 1971) and spontaneously hypertensive rats (SHR) (Hidaka & Takeya, 1972) and a hypotensive action in normotensive man (Hidaka *et al.*, 1973) and animals (Hidaka & Takeya, 1972). These anti-hypertensive and hypotensive actions of FA had been tentatively explained as due to a reduction in noradrenaline biosynthesis through the inhibition of DBH. It was at least 2–4 h after giving FA before a significant reduction in the vascular noradrenaline could be observed (Hidaka *et al.*, 1975). Although the antihypertensive and the hypotensive actions of FA were attributed to the inhibition of DBH (Hidaka *et al.*, 1974b), it is unlikely that such a slow decrease in tissue noradrenaline that is produced by this mechanism could account for the rapid hypotensive effect. The rapid decrease in blood pressure after FA is more probably related to its vasodilator action.

In an attempt to determine why the vascular smooth muscle relaxed with 5-alkylpicolinic acids and their derivatives, the effects of several blocking agents such as propranolol, atropine, aminophylline, haloperidol and ouabain were determined. As shown in Table 2, the vascular relaxations induced by 5-alkylpicolinic acids were not affected by pretreatment with propranolol or atropine. Aminophylline, an adenosine antagonist, did not affect vascular relaxations induced by the 5-alkylpicolinc acids. These results indicate that the arterial relaxations by 5-alkylpicolinic acids were not mediated through β-adrenoceptors or muscarinic receptors. However the vascular relaxations caused by 5-alkylpicolinic acid were enhanced by pretreatment with haloperidol and were attenuated by pretreatment with ouabain. In view of the fact that ouabain attenuated the relaxations produced by 5-alkylpicolinic acids, activation of the electrogenic Na^+ pump has to be considered as a possible mechanism of action. Haloperidol-induced enhancement and

Table 3 Effects of haloperidol and ouabain on various vasodilator agents

Compounds		*Control*		*Haloperidol§ (1×10^{-5} M)*		*Ouabain§ (2×10^{-7} M)*		*(1×10^{-6} M)*	
		n		n		n		n	
% Relaxation*	FA	19	60.1 ± 2.6	7	79.3 ± 5.0**	10	49.2 ± 5.4*	9	32.1 ± 5.9***
	5-pentyl PA	9	62.1 ± 3.4	8	83.3 ± 2.5***	6	43.2 ± 7.0*	5	32.1 ± 6.3***
	Br_2FA	13	73.2 ± 1.4	8	87.1 ± 2.5***	7	63.0 ± 3.9*	6	32.4 ± 5.2***
	YP-279	11	64.5 ± 2.6	8	80.4 ± 3.0***	11	54.6 ± 4.7*	6	17.4 ± 4.5***
	YP-614	15	43.1 ± 3.8	8	71.4 ± 3.1***	6	45.3 ± 1.6	6	23.8 ± 4.6*
	Isoprenaline‡	12	18.1 ± 1.6	13	25.2 ± 2.5*			12	7.6 ± 2.4**
	Dibutyryl-cyclic AMP	5	62.6 ± 2.8	5	78.2 ± 3.7*			5	24.8 ± 8.7**
	Amino-phylline	11	41.2 ± 3.0	6	55.9 ± 2.9*			5	12.1 ± 3.0***
−Log molar ED_{50}†	FA	19	3.67 ± 0.04	7	3.84 ± 0.05	10	3.83 ± 0.05	9	3.64 ± 0.05
	5-pentyl PA	9	4.07 ± 0.04	8	4.07 ± 0.11	6	3.94 ± 0.10	5	3.82 ± 0.12
	Br_2FA	13	3.87 ± 0.05	8	4.03 ± 0.07	7	3.80 ± 0.06	6	3.55 ± 0.07
	YP-279	11	3.43 ± 0.03	8	3.61 ± 0.05	11	3.59 ± 0.04	6	3.40 ± 0.03
	YP-614	15	3.33 ± 0.01	8	3.45 ± 0.03	6	3.55 ± 0.08	6	3.36 ± 0.03
	Isoprenaline‡	12	6.21 ± 0.13	13	5.91 ± 0.10			12	6.28 ± 0.09
	Dibutyryl-cyclic-AMP	5	3.48 ± 0.06	5	3.49 ± 0.08			5	3.51 ± 0.11
	Amino-phylline	11	3.46 ± 0.08	6	3.47 ± 0.07			5	3.47 ± 0.09

Abbreviations as in Table 2.

* Percentage relaxation produced by 1×10^{-3} M of each compound (with exception of isoprenaline (1×10^{-5}M)) and calculated as in Table 1.

† The ED_{50} value was obtained visually from a plot of percentage relaxation *v.* log concentration of a compound and expressed as the negative logarithm (−log molar ED_{50}). The maximum relaxation induced by the compound was taken as 100%.

‡ Preparation was exposed to phenoxybenzamine (5×10^{-6} M) for 60 min and then washed out with drug-free bathing solution before addition of isoprenaline (1×10^{-5} M).

§ Haloperidol and ouabain were added 10 min before the addition of prostaglandin $F_{2\alpha}$

n indicates the number of preparations used. Data are expressed as mean ± s.e. mean.

ouabain-induced attenuation vascular relaxation are not specific for 5-alkylpicolinic acids as they similarly affect other vasodilators such as isoprenaline, dibutyryl cyclic AMP and aminophylline. The mechanism of the effects of haloperidol and ouabain on vascular relaxation induced by 5-alkylpicolinic acids is now under investigation.

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References

ASANO, M. & HIDAKA, H. (1975). Direct effect of fusaric acid on the blood vessels. *Jap. J. Pharmac.*, **25**, suppl., 188.

BUCKNER, C.K. & SAINI, R.K. (1975). On the use of functional antagonism to estimate dissociation constants for *beta* adrenergic receptor agonists in isolated guinea-pig trachea. *J. Pharmac. exp. Ther.*, **194**, 565–574.

FURCHGOTT, R.F. & BHADRAKOM, S. (1953). Relations of strips of rabbit aorta to epinephrine, isopropylarterenol, sodium nitrite and other drugs. *J. Pharmac. exp. Ther.*, **108**, 129–143.

HIDAKA, H. & ASANO, M. (1976). Relaxation of isolated rabbit arteries by Fusaric (5-butylpicolinic) acid. *J. Pharmac. exp. Ther.*, **199**, 620–629.

HIDAKA, H., HARA, F., HARADA, N. & ASANO, M. (1974b). *In vivo* inhibition of dopamine β-hydroxylase and blood pressure. *Jap. J. Pharmac.*, **24**, suppl., 25.

HIDAKA, H., HARA, F., HARADA, N., HASHIZUME, Y. & YANO, M. (1974a). Selective inhibition of dopamine β-hydroxylase in the peripheral tissues by 5-dimethyldithocarbamylpicolinic acid: its effect on stress-induced ulcer, ethanol-induced sleep and blood pressure. *J. Pharmac. exp. Ther.*, **191**, 384–392.

HIDAKA, H., NAGASAKA, A. & TAKEDA, A. (1973). Fusaric (5-butylpicolinic) acid: its effect on plasma growth hormone. *J. clin. Endocrinol. Metab.*, **37**, 145–147.

HIDAKA, H., NAGATSU, T., TAKEYA, K., TAKEUCHI, T., SUDA, H., KOJIRI, K., MATSUZAKI, M. & UMEZAWA, H. (1969). Fusaric acid, a hypotensive agent produced by fungi. *J. Antibiot. (Tokyo) Ser. A.*, **22**, 228–230.

HIDAKA, H., SHOKA, F., HASHIZUME, Y., TAKEMOTO, N. & YAMAMOTO, M. (1975). Inhibition of dopamine β-hydroxylase in blood vessels by picolinic acid derivatives *in vitro* and their anti-hypertensive effects. *Jap. J. Pharmac.*, **25**, 515–524.

HIDAKA, H. & TAKEYA, K. (1972). Blood pressure decreased by halobutylpicolinic acid, an inhibitor of dopamine β-hydroxylase. *Nature, Lond.*, **239**, 334–335.

LEWIS, J.H. & KOESLLER, K.K. (1927). Demonstration of arterial contraction *in vitro*. *Archs intern. Med.*, **39**, 182–187.

VAN ROSSUM, J.M. (1963). Cumulative dose-response curves. II. Techniques for the making of dose-response curves in isolated organs and the evaluation of drug parameters. *Arch int. Pharmacodyn. Thér.*, **143**, 299–330.

SUDA, H., TAKEUCHI, T., NAGATSU, T., MATSUZAKI, M., MATSUMOTO, I. & UMEZAWA, H. (1969). Inhibition of dopamine β-hydroxylase by 5-alkylpicolinic acid and their hypotensive effects. *Chem. Pharm. Bull.*, **17**, 2377–2380.

TERASAWA, F. & KAMEYAMA, M. (1971). The clinical trial of a new hypotensive agent, 'fusaric acid (5-butylpicolinic acid)'. The preliminary report. *Jap. Circ. J.*, **35**, 339–357.

TODA, N. (1974). The action of vasodilating drugs on isolated basilar, coronary and mesenteric arteries of the dog. *J. Pharmac. exp. Ther.*, **191**, 139–146.

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