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The Role of Demethylbetahistine in the Depressor Response to Betahistine in the Rat (37893)

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Betahistine (2-(2-methylaminoethyl) pyridine) is a synthetic analog of histamine, and appears to have similar pharmacologic actions on the circulatory system (1). Available evidence suggests that betahistine lowers peripheral vascular resistance, and since this histamine-like drug is orally active (2–4), some interest in betahistine has been generated as a potential antihypertensive agent. Anderson and Kubicek (5) administered betahistine to pentobarbital-anesthetized dogs and examined blood flow through the basilar artery by electromagnetic flowmetry. Their findings show that betahistine increased blood flow and decreased femoral arterial blood pressure, suggesting that vascular resistance is lowered in response to intravenous betahistine.

The effect of betahistine on vascular resistance was further examined in the perfused forelimb of the dog (6). Betahistine and demethylbetahistine (2-(2-aminoethyl) pyridine), a putative metabolite of betahistine, were equipotent on a molar basis in reducing forelimb vascular resistance, and since 2-pyridylacetic acid was ineffective in altering vascular resistance, a hypothetical scheme for the metabolism of betahistine was proposed: (1) direct oxidation of betahistine by amine oxidase with eventual formation of 2-pyridylacetic acid, and (2) demethylation of betahistine to demethylbetahistine and subsequent oxidation by amine oxidase to form 2-pyridylacetaldehyde which is then converted to 2-pyridylacetic acid. More recently Bowman *et al.* (7) examined routes of betahistine metabolism and reported that betahistine or demethylbetahistine was converted to 2-pyridylacetate. These data from the rabbit support those of previous studies in the dog (6) which implicate demethylase and amine oxidase as enzymes playing a role in the metabolic conversion of betahistine to the pharmacologically inactive metabolite, 2-pyridylacetic acid.

Since available evidence suggests that there appears to be no significant difference in the action between betahistine and demethylbetahistine, and since biochemical studies suggest that betahistine may be demethylated to demethylbetahistine, the possibility remains that the pharmacological effect of betahistine may result from the combined actions of betahistine and its demethylated metabolite. In view of this, the present study was conducted to determine whether demethylbetahistine contributes to the vasodepressor response observed after intravenous administration of betahistine.

Materials and Methods. Male Wistar rats weighing between 300 and 320 g were obtained from Carworth Laboratories and employed throughout the study.

Vasodepressor responses. Rats were anesthetized with pentobarbital sodium (40 mg/kg, ip, Nembutal, Abbot Laboratories) and secured to the surgical board in the ventral position. Drugs were administered intravenously into the cannulated (PE 60) external jugular vein. Arterial blood pressure was monitored from the cannulated (PE 50) left femoral artery by means of

a Statham P23DC pressure transducer, and pressure was recorded on a Grass Model 7D polygraph. Betahistine, demethylbetahistine, and histamine (Aldrich Chemical Company) were administered in random sequence to the rat, and maximal decrease in mean arterial blood pressure was recorded. After obtaining control depressor responses, rats were pretreated with tripelennamine (Ciba 2.5 mg, iv) and vasodilatation in response to these drugs was determined. Additionally, the depressor response after betahistine administration was examined in 10 rats after pretreatment for 30 min with SKF25-A (40 mg/kg, ip).

Metabolism of betahistine in vitro. Rats were sacrificed by decapitation and livers were immediately removed, washed with ice-cold 0.02 *M* Tris–HCl buffer (pH 7.4), weighed, and homogenized with 4 vol of buffer. After homogenization with a glass–Teflon homogenizer, the whole homogenate was centrifuged at 10,000*g* for 20 min in a Sorvall RC-2B refrigerated centrifuge. The 10,000*g* supernatant fraction was used in all subsequent studies. Protein content was determined by the method of Lowry *et al.* (8) using bovine serum albumin as the protein standard.

Incubation flasks contained enzyme (10,000*g* supernatant) equivalent to 30 mg of liver protein, NADP (2 μmoles), glucose 6-phosphate (50 μmoles), glucose 6-phosphate dehydrogenase (2 IU) $MgCl_2$ (24 μmoles), betahistine (25 μmoles), or ethylmorphine (2 μmoles) and 0.5 *M* Tris–HCl buffer (pH 7.4) containing 1.15% KCl adjusted to a final volume of 5 ml. Incubations were carried out on a Dubnoff metabolic shaker in air for 30 min at 37° after a 5-min preincubation of reaction flask contents in the absence of substrate. Formaldehyde production was assayed by the procedure of Nash (9). Ethylmorphine *N*-demethylation was determined concurrently and served as a control to determine the viability of the enzyme system.

Results and Discussion. The results in Table I indicate that betahistine and its putative demethylated metabolite lower arterial blood pressure in the rat. Involvement of histamine receptors in the depressor response is suggested by the finding that the antihistamine, tripelennamine, blocks the hypotensive response observed after intravenous administration of betahistine or demethylbetahistine. Furthermore at the doses selected in this study (sufficient to decrease mean blood pressure approximately 50 mmHg), histamine appears about 20 times more potent than either betahistine or demethylbetahistine. Greater pharmacological activity of histamine when compared to these pyridylalkylamines has also been observed by Konzett *et al.* (6) who reported that histamine is about 200 times more

TABLE I. The Effect of Tripelennamine on the Depressor Response to Betahistine or Demethylbetahistine in the Rat.

Drug	Dose[a] (μmole)	Depressor response[b] decrease in mean pressure (mmHg)	
		Tripelennamine[c]	
		Before	After
Betahistine	0.717	57 ± 5[d]	7 ± 6
Demethylbetahistine	0.820	48 ± 4	4 ± 1
Histamine	0.045	55 ± 2	10 ± 4

[a] Doses were selected on the basis of causing similar magnitudes in depressor responses.

[b] Maximal decrease in mean blood pressure after intravenous drug administration.

[c] Tripelennamine (2.5 mg, iv) was given to rats and depressor responses were examined 5–15 min after administration of the antihistamine.

[d] Mean values ± standard error obtained from 10 rats.

active in decreasing vascular resistance in the perfused dog forelimb. Moreover, in this connection, betahistine is about 8% as active as histamine on isolated intestine (10), and more recently the effect of betahistine on gastric acid secretion in the conscious dog has been reported to be roughly 20% of the maximal response to histamine (11).

In light of the observation that betahistine and demethylbetahistine activate hypotensive responses in the rat, and of the possibility that betahistine may be metabolized to demethylbetahistine, we reasoned that the depressor response observed after intravenous administration of betahistine may, in fact, result from the combined actions of betahistine and demethylbetahistine. Therefore, rats were pretreated with SKF525-A in an effort to inhibit hepatic microsomal *N*-demethylase activity, and the depressor response to betahistine was then examined. The data in Table II indicate that SKF525-A pretreatment caused no alteration of the betahistine-induced depressor response measured as a decrease in arterial blood pressure or as the time required for total drug action. These findings would support the contention that the systemic arterial pressure response to intravenous administration of betahistine results primarily from the action of the parent compound. Furthermore, if the pretreatment regimen of SKF525-A (40 mg/kg, ip 30 min) sufficiently blocked *N*-demethylase activity, then the findings reported in Table II suggest that the enzymatic conversion of betahistine to demethylbetahistine may be lacking in rat liver. In view of this suggestion an attempt was made to determine betahistine *N*-demethylase activity *in vitro*. There was no detectable betahistine *N*-demethylase activity in saline- or phenobarbital-pretreated rats, indicating that betahistine is not metabolized to demethylbetahistine in this species (Table III). That the 10,000*g* supernatant system was capable of catalyzing the demethylation reaction is indicated by the finding that ethylmorphine was demethylated and that this demethylation was stimulated approximately 4-fold in the phenobarbital-pretreated rat. These results indicating no demonstrable betahistine *N*-demethylase activity coupled with the finding that the vasodepressor response to betahistine was unaltered by SKF525-A pretreatment favor the contention that demethylbetahistine does not contribute to the depressor action observed after intravenous administration of betahistine to the rat. Additionally, since betahistine does not appear to be demethylated in the rat, the results suggest that the primary route of metabolism of this drug may involve direct deamination of betahistine with ultimate

TABLE II. Effect of SKE25-A Pretreatment on the Magnitude and Duration of the Depressor Action of Betahistine.

	Betahistine[a] depressor response	
Pretreatment	Magnitude[b] (mmHg)	Duration[c] (sec)
Saline	56 ± 5[d]	32 ± 3
SKF25-A[e]	60 ± 3	36 ± 3

[a] Given intravenously (0.717 μmole total dose).

[b] Maximal decrease in mean blood pressure after betahistine.

[c] Time required for return to predrug baseline or new stable baseline of mean blood pressure.

[d] Represent mean values ± standard error obtained from 10 rats.

[e] SKF25-A was administered (40 mg/kg ip) and the depressor response to betahistine was examined 30 min after drug injection.

TABLE III. Lack of *N*-Demethylation of Betahistine by Rat Liver Homogenates.[a]

	N-Demethylase activity (nmoles HCHO formed/mg protein/30 min) substrate	
Pretreatment	Betahistine	Ethylmorphine
Saline	—[b]	20.4 ± 1.5[c]
Phenobarbital[d]	—[b]	77.3 ± 4.8[c]

[a] 10,000*g* supernatant.

[b] No detectable *N*-demethylase activity.

[c] Mean values ± standard deviation of four rats (three determinations for each rat).

[d] Phenobarbital administered (80 mg/kg, ip) for 3 days.

formation of the pharmacologically inactive metabolite 2-pyridylacetic acid.

Summary. Betahistine and demethylbetahistine lower arterial blood pressure in the rat, and the depressor response was blocked by pretreatment with the antihistamine, tripelennamine. Rats pretreated with SKF-525-A exhibited no modification of the depressor action of betahistine. No detectable betahistine *N*-demethylase activity was observed in rat liver homogenates. The data do not support the contention that demethylbetahistine contributes to the overall depressor response observed after intravenous administration of betahistine, and suggest that direct deamination of betahistine may represent the major pathway of enzymatic degradation of this drug in the rat.

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