

## Vecuronium Inhibits Histamine N-Methyltransferase

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Although there have been clinical reports of significant hypotension and flushing associated with the use of vecuronium, it produces minimal cardiovascular effects in the vast majority of patients. In addition, there is no evidence that vecuronium stimulates the release of histamine. The authors performed *in vitro* kinetic studies to determine the effect of vecuronium on histamine N-methyltransferase (HNMT), the primary catabolic enzyme for histamine in humans. They also examined plasma from patients who had received vecuronium (0.1 or 0.2 mg/kg) to determine whether clinically used concentrations of the drug could inhibit HNMT. It was determined that vecuronium is a strong inhibitor of HNMT; apparent  $K_i = 1 \mu\text{M}$ . The inhibition is competitive with respect to methyl-donor and noncompetitive with respect to histamine. Vecuronium, in doses  $\geq 0.1 \text{ mg/kg}$ , may delay the metabolism of histamine by HNMT *in vitro*. (Key words: Histamine: Assay; metabolism. Neuro-muscular relaxants: vecuronium.)

VECURONIUM IS A nondepolarizing muscle relaxant having an intermediate duration of action and few hemodynamic side effects.<sup>1-4</sup> Its administration is not followed by increased plasma histamine levels in animals or humans.<sup>5,††</sup> There have been, however, recent reports of severe hypotension and flushing following the administration of vecuronium.<sup>6-8</sup> The etiology of these events is unclear.

Muscle relaxants can inhibit the activity of histamine-N-methyltransferase (HNMT), an enzyme responsible for inactivating histamine in humans.<sup>9,‡‡</sup> Significant in-

hibition of HNMT may exacerbate the cardiovascular effects of histamine release caused by many anesthetic drugs.

Our *in vitro* kinetic studies used purified HNMT to evaluate the role of vecuronium in HNMT inhibition, and to characterize the nature of this inhibition. We also examined plasma from patients who had received vecuronium (0.1 or 0.2 mg/kg) to ascertain whether vecuronium could inhibit HNMT in a clinical setting.

### Materials and Methods

#### REAGENTS

Histamine dihydrochloride and bovine serum albumin (radioimmunoassay grade) were purchased from Sigma (St. Louis, MO). Bis (2-ethylhexyl) hydrogen phosphate was obtained from Eastman Kodak (Rochester, NY). Econofluor and S-adenosyl-L-[3H-methyl]-methionine (SAM), 13.6-14.7 Ci/mM were purchased from New England Nuclear (Boston, MA). New England Nuclear's lot analysis of the SAM showed  $\geq 95\%$  purity and radiochemical purity. Pure vecuronium bromide and pancuronium were the gifts of Organon (West Orange, NJ). All other chemicals were of reagent quality or better. Pipecuronium was obtained from Gedeon Richter Chemical Works (Budapest).

#### PREPARATION AND STORAGE OF CHEMICALS

Histamine dihydrochloride stock solutions, 0.1 mg/ml, prepared and stored in 0.2 N HCl, were diluted with distilled water in polypropylene tubes to yield the desired concentrations. SAM was diluted in 1 M potassium phosphate-8 mM EDTA, pH 7.8, and 0.1% bovine serum albumin, to the desired concentration. HNMT was diluted 1:1000 with 0.1% bovine serum albumin. Vecuronium was dissolved and diluted in 0.002 N HCl.

#### PREPARATION OF HISTAMINE N-METHYLTRANSFERASE

HNMT was purified from rat kidneys 260-fold by ion exchange and molecular exclusion chromatography according to the method used by Bowsher *et al.*<sup>10</sup>

#### METHODS

HNMT activity was assayed as N-[methyl-3H] histamine produced using a modification of the method of Bowsher *et al.*<sup>10-12</sup> The incubation volume was 100

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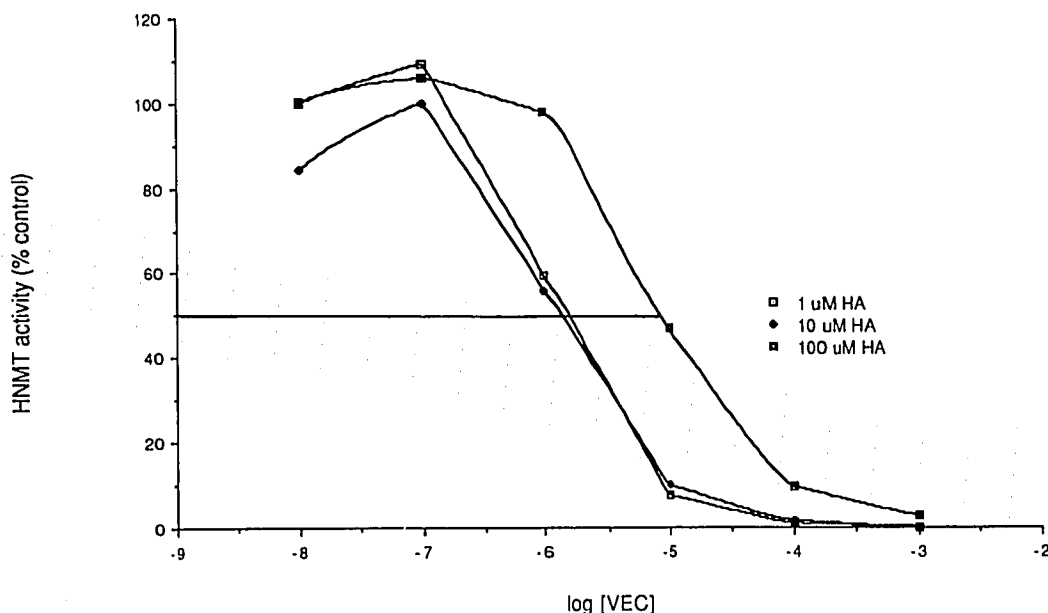
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†† Basta JS, Savarese JJ, Ali HH, Sunder N, Moss J, Gionfriddo M, Embree P: Vecuronium does not alter serum histamine within the clinical dose range (abstract). ANESTHESIOLOGY: 59:A273, 1983

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FIG. 1. Inhibition of HNMT by vecuronium. The ordinate is HNMT activity expressed in percentage of total activity. The abscissa is the logarithmic molar concentration of vecuronium. Curves are shown for 1, 10, and 100  $\mu\text{M}$  histamine. Solid lines are the interpolation of average values.



$\mu\text{l}$ :25  $\mu\text{l}$  of buffered albumin containing SAM, 25  $\mu\text{l}$  of histamine working solution, 25  $\mu\text{l}$  of vecuronium working solution, and 25  $\mu\text{l}$  of HNMT working solution. The components were sequentially added to the tubes on ice and the mixture incubated for 20 min at 37° C. Preliminary studies showed that, under these conditions, the reaction rates were linear for at least 30 min, at the highest and lowest substrate concentrations used, allowing us to determine  $K_i$  with equations described below. The reaction velocity was measured as disintegrations per minute/minute (dpm/min). The concentration of vecuronium varied from  $10^{-8}$  to  $10^{-3}\text{M}$ . Histamine concentrations were 1.0;1.25;2.5;5.0;10.0; 100.0  $\mu\text{M}$ , and [SAM] fixed at 1.0  $\mu\text{M}$ . In other assays, histamine was fixed constant at 10  $\mu\text{M}$  and [SAM], 0.5;1.0;2.0;4.0  $\mu\text{M}$ . Blanks containing no histamine were included in all assays. The assays were performed in quadruplicate.

Results were plotted using Lineweaver-Burk double reciprocal plots that were analyzed by linear regression. In the case of competitive inhibition,  $K_i$  values were derived from secondary plots of slopes of the Lineweaver-Burk plots versus inhibitor concentration. In the second case, that of noncompetitive inhibition,  $K_i$  values were derived from secondary plots of the intercepts ( $1/V_{\text{max,apparent}}$ ) of Lineweaver-Burk plots versus inhibitor concentration.<sup>15</sup>

#### CLINICAL STUDIES

After obtaining informed consent, 19 patients scheduled for elective surgery were anesthetized with  $\text{N}_2\text{O}/\text{halothane}/\text{O}_2$  and the trachea was intubated without muscle relaxants. Ventilation was controlled ( $\text{PaCO}_2$

=  $40 \pm 3$  mmHg) and end-tidal halothane was maintained at 0.7% as monitored by mass spectroscopy. Subjects were randomly assigned to receive 0.1 mg/kg (group 1; N = 10) or 0.2 mg/kg (group 2; N = 9) of vecuronium. Arterial plasma samples were obtained before, and at 2, 5, and 10 min after administering vecuronium. Histamine levels were measured in duplicate with duplicate internal standards using a sensitive HNMT-base radioenzymatic assay.<sup>11</sup> The assays were performed by individuals blinded to the original design. Data were analyzed by paired and unpaired *t* tests.

#### Results

Vecuronium inhibits HNMT; the apparent  $K_i$  is 1  $\mu\text{M}$  (fig. 1). The curve for 100  $\mu\text{M}$  histamine is shifted with approximately one order of magnitude of inhibitor concentration to the right. The inhibition obeys the equation:

$$1/V = (K_m/V_{\text{max}})(1 + [I]/K_i)(1/[S]) + 1/V_{\text{max}},$$

where  $V$  is the rate,  $V_{\text{max}}$  the highest rate at a given concentration of vecuronium,  $[I]$  the concentration of vecuronium,  $K_i$  the inhibitor constant,  $K_m$  the Michaelis constant, and  $[S]$  the SAM concentration. This may be transformed to:

$$1/V = (K_m/V_{\text{max}})(1 + [I]/K_i)(1/[S]) + (1/V_{\text{max}})(1 + [I]/K_i),$$

where  $[S]$  is the histamine concentration. Lineweaver-Burk plots demonstrate that inhibition is competitive with respect to SAM ( $K_i = 1.4 \mu\text{M}$ ) (fig. 2) and noncompetitive with respect to histamine ( $K_i = 1.2 \mu\text{M}$ ) (fig. 3).

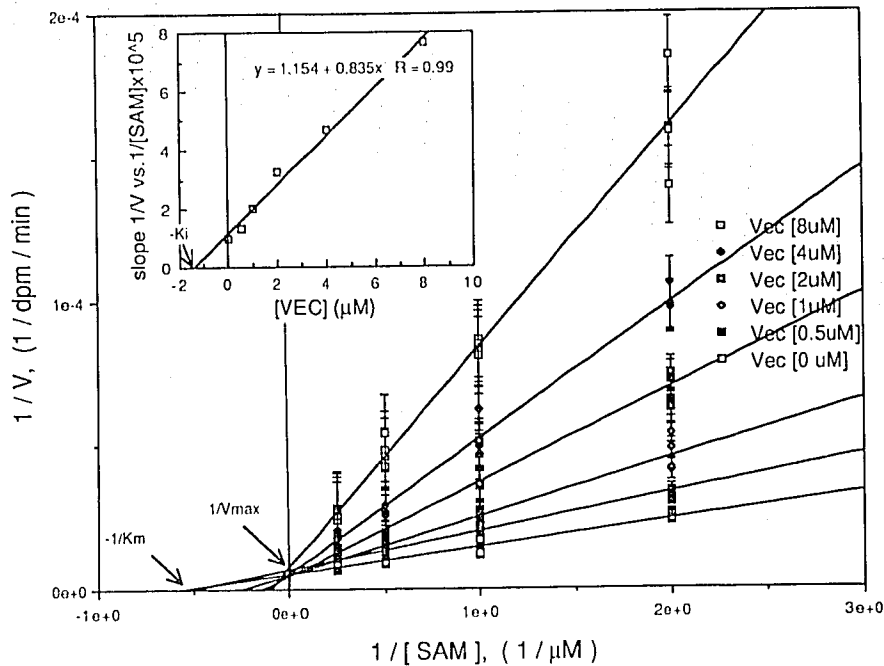


FIG. 2. Inhibition of HNMT by vecuronium. Lineweaver-Burk plot:  $1/V$  versus  $1/[SAM]$  in the presence of different vecuronium concentrations. Inset: Replots of slopes of the main plots versus vecuronium concentrations. We used linear regression to obtain points to plot all curves. Bars represent standard error of the data.

Kinetic analysis of pancuronium and pipecuronium reveal  $K_s$  of 2 and 33  $\mu\text{M}$ , and their inhibition is also noncompetitive with respect to histamine. The neuromuscular blocking potency on a molar basis of the three steroidal muscle relaxants correlates negatively with their  $K_i$  values ( $R = 1.0$ ).

The efficiency of N-methylation of histamine by HNMT was significantly decreased in the plasma of patients who had received vecuronium. Although the enzymatic efficiency tended to recover 10 min after vecuronium administration, it still was significantly de-

pressed compared to initial levels (table 1). The samples from both groups, obtained prior to administering vecuronium, did not differ statistically. The measured histamine concentrations of patients from group 1 and group 2 differed significantly ( $0.025 < P \leq 0.05$ ) only at 2 min after vecuronium was administered.

## Discussion

HNMT and diamine oxidase are two enzymes that catalyze histamine degradation. In humans, ring meth-

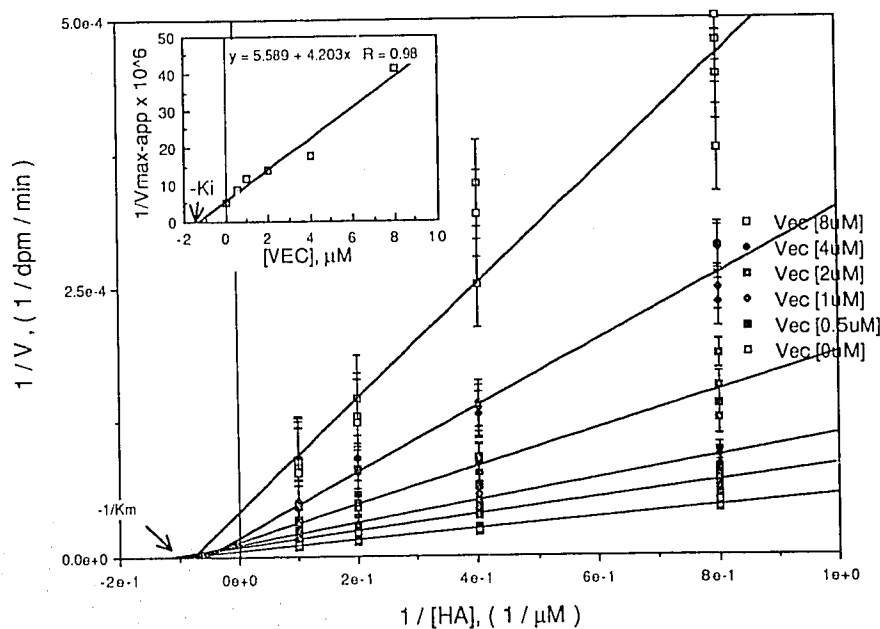


FIG. 3. Inhibition of HNMT by vecuronium. Lineweaver-Burk plot:  $1/V$  versus  $1/[\text{histamine}]$  in the presence of different vecuronium concentrations. We used linear regression to obtain points to plot all curves. (Vecuronium concentrations are the same as shown in figure 2.) Inset:  $1/V_{\text{max-apparent}} \times 10^{-6}$  versus vecuronium concentrations.

TABLE 1. Efficiency of Histamine N-Methyltransferase Before and After Vecuronium Administration. Values Expressed as Mean Counts Per Minute Per Picogram  $\pm$  SD

Dose	0 Min	2 Min	5 Min	10 Min
0.1 mg/kg	87.7 $\pm$ 24.6	72.7 $\pm$ 18.4*	75.5 $\pm$ 23.2†	77.4 $\pm$ 22.8†
0.2 mg/kg	97.8 $\pm$ 21.3	58.4 $\pm$ 14.1‡	66.4 $\pm$ 15.3‡	71.1 $\pm$ 17.4‡

\* Significantly different ( $.01 < P \leq .025$ ) from control.† Significantly different ( $.0005 < P < .005$ ) from control.‡ Significantly different ( $P \leq .0005$ ) from control.

ylation by HNMT appears to predominate over oxidative deamination by diamine oxidase (DAO). The alternative pathway becomes significant only in women with ovarian carcinoma or in women in the third trimester of pregnancy.<sup>14</sup> These enzymes are widely distributed throughout the various organs, though they are found in greater concentrations in the liver and the kidneys, which accounts for the short plasma half-life of infused histamine and the small amount, 1%, found in urine.<sup>14,15</sup>

We have demonstrated that vecuronium is a potent inhibitor of HNMT *in vitro*. Furthermore, our observations from the clinical samples are consistent with the kinetic results. The inhibition is competitive with respect to the cosubstrate S-adenosyl-methionine, but noncompetitive with respect to histamine. The maximum rate of the reaction cannot be restored even by infinitely high concentrations of the substrate in the presence of a noncompetitive inhibitor. Thus, high plasma histamine levels, such as may occur during an anaphylactic reaction, will not overcome the inhibition.

The pharmacokinetic data of previous studies showed that the volume of distribution at steady state of vecuronium varied between 179–270 ml/kg in patients without hepatic or renal failure.<sup>16–18</sup> When intubating doses of vecuronium (0.089–0.12; 0.14–0.28; 0.15 mg/kg) were administered in bolus or rapid infusion, the peak plasma concentrations were 1.3–4  $\mu$ g/ml (2.02–6.30  $\mu$ M); these levels decreased to 0.25–0.4  $\mu$ g/ml (0.39–0.63  $\mu$ M) by the end of distribution ( $T_{1/2\alpha}$  ranged between 3.9 and 12.7 min).<sup>16,17</sup>§§ We derived the percent HNMT inhibition from the vecuronium HNMT activity curve where SAM and histamine were both approximately 1  $\mu$ M. The inhibition during distribution for a 0.1 mg/kg dose of vecuronium was 26–38%; for a 0.2 mg/kg dose, it was 45–54%. Vecuronium administered at ED<sub>95</sub><sup>19</sup> for neuromuscular blockade may still cause 20% reduction in HNMT activity.

Our data indicate that high doses of vecuronium could interfere with the HNMT-based radioenzymatic

measurement of histamine. This effect should persist for at least 10 min (table 1). Increasing the concentration of SAM up to 4  $\mu$ M minimizes the inhibition (fig. 2), but cannot totally eliminate possible errors in determining histamine levels.

We do not understand why vecuronium causes competitive inhibition of the SAM binding site to HNMT. Other studies have demonstrated that nondepolarizing muscle relaxants inhibit plasma cholinesterase in direct proportion to the clinical potency.<sup>20</sup> It is possible that the amino acid sequence of the SAM binding site of HNMT is homologous to that of the acetylcholine site of plasma cholinesterase.

The extent to which the inhibition of HNMT activity by vecuronium will reduce histamine metabolism *in vivo* is not established. Several studies report significant hypotension following vecuronium administration, but there was no evidence of histamine release. One possible explanation is that a dose of vecuronium sufficient for tracheal intubation may inhibit HNMT to an extent not previously appreciated.

We have demonstrated that three steroidal neuromuscular relaxants (vecuronium, pancuronium, and pipecuronium) are all inhibitors of HNMT with  $K_i$  values of 1, 2, and 33  $\mu$ M, respectively. When distribution kinetics and  $K_i$  are considered, our calculations suggest that only vecuronium could achieve a level in plasma at which the inhibition should be clinically relevant. Vecuronium in doses 0.1–0.15 mg/kg may elicit more than 50% inhibition of HNMT transiently, but cause a 25–50% inhibition of HNMT lasting 10–14 min after administration. Pancuronium (0.06 mg/kg) would be expected to show a 25% inhibition of HNMT for seconds. Pipecuronium does not inhibit HNMT in clinical doses.‡‡

Several issues complicate efforts to interpret the clinical significance of this study. The neuromuscular blocking agents, which are highly water soluble, may have limited access to HNMT, which is found predominantly in cytoplasm. Numerous pharmacologic inhibitors of HNMT, including vecuronium, might be administered without apparent hemodynamic changes in the absence of a histamine-releasing drug or procedure. Under normal conditions, the enzymatic mechanisms responsible for inactivating plasma histamine are ex-

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tremely efficient, although both pathways may be inhibited by neuromuscular relaxants. Inhibition of histamine catabolism by DAO inhibitors results in increased plasma histamine levels and faster mortality in dogs with superior mesenteric artery occlusion and reperfusion.<sup>21,22</sup> Although other relaxants, including d-tubocurarine, pancuronium, and alcuronium, can inhibit DAO *in vitro*, there is no evidence that vecuronium inhibits this alternative pathway, or of its clinical relevance.<sup>23</sup> Nonetheless, it is apparent from animal experiments that, when histamine catabolism (DAO) is blocked, there are increased plasma histamine levels and lethality. This inhibition of HNMT by vecuronium merits further study.

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