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INHIBITION OF HISTAMINE *N*-METHYLTRANSFERASE (HNMT) *IN VITRO* BY NEUROMUSCULAR RELAXANTS

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Abstract—There have been reports of hypotension and flushing following vecuronium administration. The etiology of these symptoms, which are similar to those of histamine release, is not clear. The steroidal neuromuscular relaxants (NMRs), unlike muscle relaxants structurally similar to curare, have been shown not to cause histamine release after the administration of typical clinical doses. Histamine levels in plasma reflect a balance between release and catabolism. In humans, histamine *N*-methyltransferase (HNMT) is the enzyme primarily degrading for histamine. Therefore, we performed *in vitro* kinetic studies of purified HNMT to determine the effects of the steroidal and curare-like NMRs and also of gallamine on histamine catabolism. We demonstrated that all NMRs tested were inhibitors of HNMT *in vitro*. The inhibition was competitive with respect to the cosubstrate *S*-adenosyl-*L*-[^{3}H-*methyl*] methionine, and noncompetitive with respect to histamine. The rank order of inhibition was vecuronium>pancuronium>gallamine>*d*-tubocurarine>metocurine>atracurium>pipecuronium, with K_i values ranging from 1.2 to 44.8 μM. Our data suggest that HNMT-based radioenzymatic assays for histamine should be susceptible to inhibition by concurrent use of NMRs, particularly vecuronium.

There have been reports of unexplained hypotension and flushing following the administration of vecuronium [1–3]. The etiology of these effects is unclear. The steroidal neuromuscular relaxants (NMRs), unlike the muscle relaxants that are structurally similar to curare, do not cause histamine release after administration of typical clinical doses [4–6]. Intradermal administration of steroidal NMRs, moreover, may produce a wheal-and-flare reaction without hemodynamic changes [7].

In vivo, histamine is cleared from plasma by two enzymes, histamine-*N*-methyltransferase (HNMT) and diamine oxidase (DAO). The clearance of histamine from plasma is very efficient with both enzymatic pathways intact [8]. Blocking the action of DAO in experimental animals potentiates the hemodynamic sequelae which follow the infusion of histamine or its endogenous release associated with intestinal ischemia and reperfusion [9, 10]. Inhibiting HNMT activity in human plasma may similarly accentuate the effects of histamine release.

We studied the inhibition of rat kidney HNMT by the steroidal and curare-like NMRs in order to evaluate their potential effects on histamine catabolism. We utilized *in vitro* kinetic studies of purified HNMT to characterize the nature of the inhibition. We also examined the effects of gallamine, an NMR of unrelated structure, and those of dexamethasone, a corticosteroid having no known NMR properties.

METHODS

Reagents. Histamine dihydrochloride, bovine serum albumin (radioimmunoassay grade), gallamine triethiodide, *d*-tubocurarine chloride, and dexamethasone were purchased from the Sigma Chemical Co. (St. Louis, MO). Bis-(2-ethylhexyl) hydrogen phosphate was obtained from Eastman Kodak (Rochester, NY). Econofluor and *S*-adenosyl-L-[^{3}H-*methyl*] methionine [^{3}H]SAM, 13.6 to 14.7 Ci/mmol) were purchased from New England Nuclear (Boston, MA). The lot analysis of the [^{3}H]-SAM by New England Nuclear showed ≥ 95% purity and radiochemical purity. Metocurine iodine injection, USP, was purchased from Eli Lilly (Indianapolis, IN). Arduan® (pipecuronium bromide) was the gift of Gedeon Richter Chemical Works, Ltd. (Budapest, Hungary). Pure atracurium besylate was the gift of the Burroughs Wellcome Co. (Research Triangle Park, NC). Pure vecuronium bromide and pancuronium bromide were the gifts of Organon (West Orange, NJ). All other chemicals were of reagent quality or better.

Preparation and storage of chemicals. Histamine dihydrochloride stock solutions, 0.1 mg/ml, prepared and stored in 0.2 N HCl, were diluted in polypropylene tubes with distilled water 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. Dexamethasone and vecuronium bromide were dissolved and diluted in 0.002 N HCl, the other drugs in distilled water.

Preparation of histamine N-*methyltransferase.* HNMT was purified 260-fold from rat kidneys by ion

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exchange and molecular exclusion chromatography, according to the method of Bowsher *et al.* [11].

HNMT activity was assayed as *N*-[*methyl*-^{3}H]-histamine produced using a modification of the method of Bowsher *et al.* [11, 12]. The incubation volume was 100 μl: 25 μl of buffered albumin containing SAM, 25 μl of histamine working solution, 25 μl of NMRs working solution and 25 μl of HNMT working solution. The components were sequentially added to the tubes on ice, and the mixture was then incubated for 20 min at 37°. Preliminary studies showed that, under these conditions, the reaction rates were constant for at least 30 min, at the highest and lowest substrate concentrations used, allowing us to determine inhibitor constants (K_i) as previously described [13]. At histamine concentrations of 1, 10 and 100 μM and a SAM concentration of 1 μM, HNMT activity was calculated for each tested concentration of NMRs or dexamethasone as a percentage of total activity. The concentrations of NMRs varied from 10^{-8} to 10^{-3} M, and those of dexamethasone from 0.6×10^{-9} to 0.6×10^{-4} M.

To obtain further information about the character of the inhibition, histamine concentrations were varied at 1.25, 2.5, 5.0, and 10.0 μM and SAM was fixed at 1.0 μM. In other assays, histamine was fixed at 10 μM and SAM varied at 0.5, 1.0, 2.0, and 4.0 μM. The reaction velocity was measured as dpm/min. The concentrations of vecuronium and pancuronium varied from 5×10^{-7} to 8×10^{-6} M, pipecuronium from 7.5×10^{-6} to 10^{-4}, *d*-tubocurarine chloride from 7.5×10^{-7} to 10^{-5}, and the concentrations of the other drugs from 10^{-6} to 10^{-7} M. Blanks containing no histamine were included in all assays. The determinations of each data point were run in quadruplicate, and each data point was confirmed by a duplicate experiment as part of the matrix.

Results were plotted as HNMT activity (% of the control) against the logarithm of the inhibitor concentration. Double-reciprocal plots were used to diagnose the nature of inhibition; K_i values were derived from secondary plots of slopes of the Lineweaver–Burk plots *versus* inhibitor concentration in the case of competitive inhibition and from those of $1/V$-axis intercepts of Lineweaver–Burk plots *versus* inhibitor concentration in the case of noncompetitive inhibition [14]. Data are presented as mean ± standard deviation for quadruplicate samples run as a cohort.

RESULTS

All NMRs, but not dexamethasone, inhibited the action of HNMT (Table 1). The curve for 100 μM histamine was shifted with approximately one order of magnitude of inhibitor concentration to the right of those for 1 and 10 μM histamine. Lineweaver–Burk plots demonstrated that inhibition was competitive with respect to SAM and noncompetitive with respect to histamine for all the muscle relaxants tested (Figs. 1 and 2). K_i values are listed in Table 2. We found the rank order of inhibition with respect to histamine to be: vecuronium>pancuronium>gallamine>*d*-tubocurarine >metocurine>atracurium>pipecuronium.

Table 1. Summary of inhibition of HNMT activity by nondepolarizing neuromuscular relaxants

Drugs	% Inhibition of HNMT activity at drug concentrations (M) of				
	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}
Vecuronium	98	93	42	0	0
Pancuronium	99	94	43	0	0
Pipecuronium	84	35	0	0	0
d-Tubocurarine	96	77	18	7	0
Metocurine	91	58	22	9	0
Atracurium	90	52	10	0	0
Gallamine	93	64	10	0	10

Values for percent inhibition of HNMT activity were taken from assays where histamine and SAM were both 1 μM. The control reaction rate was 6 nmol/hr/mg protein.

DISCUSSION

Histamine levels in plasma reflect a balance between release and catabolism. Numerous clinical and laboratory studies document release of histamine by curare and similar drugs [15].

We showed that NMRs of the structural groups tested were inhibitors of rat kidney HNMT *in vitro*. The inhibition was competitive with respect to the cosubstrate SAM, and noncompetitive with respect to histamine. The rank order of inhibition was vecuronium > pancuronium > gallamine > *d*-tubocurarine >metocurine>atracurium>pipecuronium.

A previous report was published on the inhibition of HNMT by relaxants. The order of inhibition was alcuronium>pancuronium>*d*-tubocurarine = gallamine>decamethonium>succinylcholine [16]. The authors postulated that the NMRs were analogues to histamine-containing quaternary ammonium groups. The ammonium moiety of these drugs was presumed to be the structural prerequisite for inhibition of HNMT activity. Differences in potencies were thought to be due to differences in the rigidity of the molecules. It was concluded that drugs containing a quaternary ring nitrogen atom with only one side chain should be better inhibitors than drugs with the quaternary nitrogen atom incorporated in the straight chain and three methyl groups attached. It was further expected that drugs with one ammonium group should be less potent inhibitors of HNMT.

The muscle relaxant alcuronium and several antimalarial drugs of the animopyridine class have been demonstrated recently to be competitive inhibitors of HNMT [17]. However, since the authors did not carry out a full kinetic analysis of HNMT inhibition by alcuronium in which both the histamine and SAM concentrations were varied independently, it is uncertain with which cosubstrate alcuronium competes. All the neuromuscular blocking drugs in our study were competitive with respect to SAM. Antimalarial drugs, however, have been shown to inhibit HNMT competitively with respect to histamine [18, 19].

Quaternary or tertiary ammonium groups in NMR molecules are necessary to permit binding to chol-

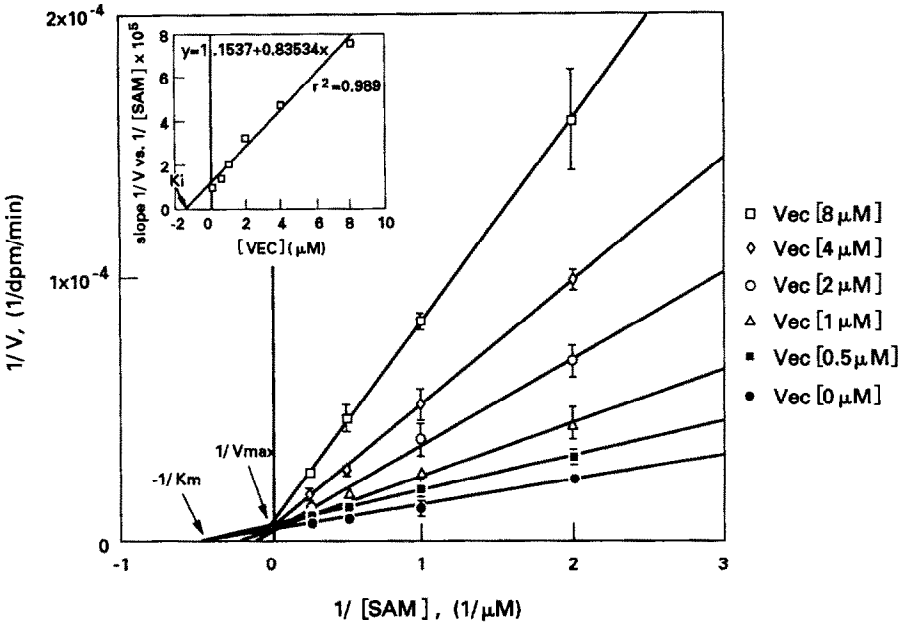

Fig. 1. Example of competitive inhibition of HNMT by neuromuscular relaxants. Lineweaver–Burk plots: 1/velocity ($1/V$) versus 1/[SAM] in the presence of different vecuronium concentrations. *Inset*: Slopes of the main plots versus [vecuronium]. All curve fits are linear regression. Bars represent SD of the data for quadruplicate samples run as a cohort.

inergic receptors; two such groups must be present for neuromuscular blockade [20]. Our study confirmed that these sites are also required to inhibit HNMT, but any structural analogy should be between SAM and NMRs, rather than between histamine and NMRs. Our kinetic analysis led us to seek features similar to adenine in our structure–activity relationship of HNMT inhibition by these compounds. We assume that the structural feature conferring maximum inhibition of HNMT is two or more

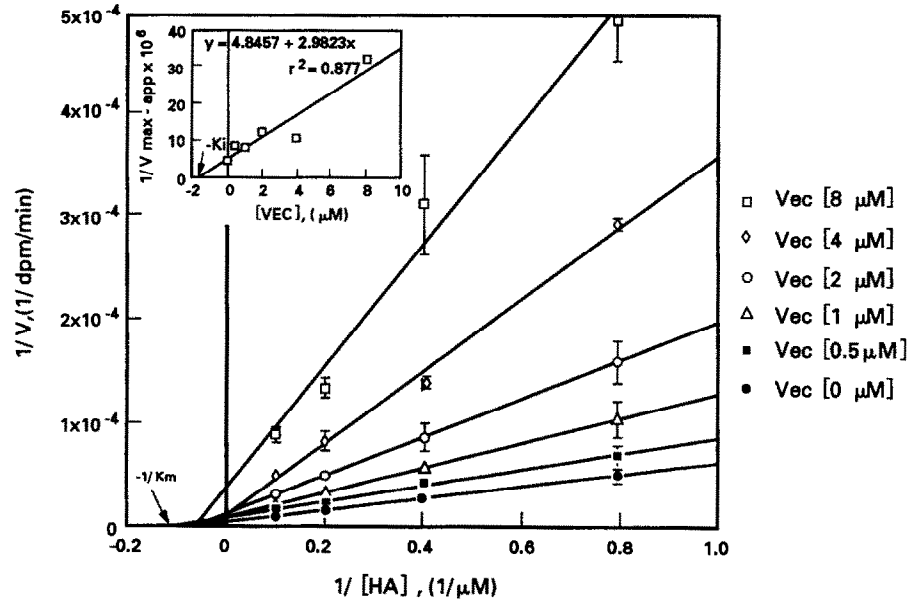

Fig. 2. Example of noncompetitive inhibition of HNMT by neuromuscular relaxants. Lineweaver–Burk plots: $1/V$ versus 1/[histamine] in the presence of different vecuronium concentrations. *Inset*: $1/V$-axis intercepts of main plots versus [vecuronium]. All curve fits are linear regression. Bars represent SD of the data for quadruplicate samples run as a cohort.

Table 2. Inhibition constants of neuromuscular relaxants for inhibition of HNMT activity

Drug	K_i with varied histamine (μM)	K_i with varied SAM (μM)
Vecuronium	1.2	1.4
Pancuronium	2.7	1.1
Pipecuronium	44.8	15.5
d-Tubocurarine	8.2	6.3
Metocurine	14.8	
Atracurium	16.3	
Gallamine	7.2	

K_i values were obtained from the secondary plots of slopes and $1/V$-axis intercepts of Lineweaver–Burk plots.

cationic amino functional groups separated about 10 Å by a planar carbon skeleton. Further separation of the ammonium functionalities and deviation from the planarity of the carbon skeleton decrease the inhibitory potency. These structural requirements overlap those of neuromuscular blocking drugs but not of the classical antihistamines. The antihistamines have been shown to compete with histamine and not with SAM when inhibiting HNMT [18] and do not affect endogenous histamine levels [21–24].

The neuromuscular blocking potency of NMRs within each structural group correlated negatively with their K_i values (Fig.3). The potency of neuromuscular relaxation was very similar within the steroidal group. HNMT blockade appeared independent of neuromuscular blocking potency in this group. The K_i values for histamine of vecuronium and pancuronium were very much alike: 1.2 and 2.7 μM. Vecuronium is simply pancuronium less an *N*-methyl group in the ring A. The nitrogen atom is protonated at physiologic pH; the absence of a methyl group slightly reduced the size of the molecule but did not affect its neuromuscular blocking activity or inhibition of HNMT. Pipecuronium is also a pancuronium analogue, with dimethyl quaternary distal nitrogens on the piperazino rings which are attached at the 2 and 16 positions of the androstene skeleton. The loss of an acetylcholine-like structure, the increase in the size of the molecule, and the considerably longer distances between charged nitrogens do not alter its relaxant potency, but produced a 20- to 40-fold reduction in the potency of HNMT inhibition. The compounds in the curare-like group show a less planar structure than those in the steroidal group. Like vecuronium, *d*-tubocurarine is a monoquaternary compound with a tertiary amine group, protonated at physiologic pH. Metocurine, the methylation product of *d*-tubocurarine, has twice its neuromuscular blocking potency, but it was only half as potent as an inhibitor of HNMT. Atracurium, with greater molecular weight and longer inter-nitrogen distance, has a slightly greater potency as a neuromuscular blocker, and a potency of HNMT inhibition close to that of metocurine. Gallamine is structurally different from both steroidal curare-form relaxants. The relationship of pK_i versus -log[EC] would not be expected to, and does not, lie on either of the defined lines. Gallamine has three quaternary nitrogens on side chains of a benzene ring. Its structure is less rigid than the other non-depolarizing NMRs; the distances between the charged nitrogens and the quaternary nitrogens and the carbonyl groups are short in gallamine. Succinylcholine, with its small size, flexibility, and straight chain structure, was the least potent inhibitor of HNMT ($K_i > 1$ mM, unpublished data from our study).

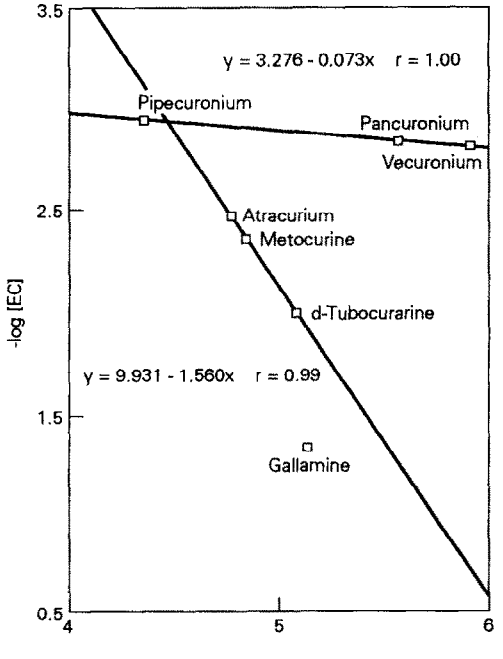

Fig. 3. Correlation of the clinical potency of the neuromuscular relaxants and HNMT inhibition. Ordinate: -log[EC], where EC equals the ratio of the equipotent doses of NMRs [15, 25] to their molecular weight. Abscissa: pK_i of NMRs. Curve fits are linear regression. Both steroidal and curare-form NMRs showed negative correlation of the potency of neuromuscular relaxation and potency of HNMT inhibition with different slopes.

In summary, the factors that increased the potency of HNMT inhibition were the distance between quaternary nitrogen and the carbonyl group, the distance between the charged nitrogens, planarity, rigidity, and optimum size of the molecule.

While there is little question that NMRs inhibited rat kidney HNMT *in vitro*, the clinical relevance of

Table 3. Predicted HNMT inhibition based on K_i values and previously determined pharmacokinetic and pharmacodynamic data

Studies	Ref.		Doses given in bolus or rapid infusion (mg/kg)	Peak concentration (μM)	Concentration at the end of distribution (μM)	HNMT inhibition in distribution phase (% control)	K_i (μM)
Fahey *et al.*	[27]	Vecuronium	0.28	7.8	1.3	49–85	1.6
Sohn *et al.*	[40]		0.15	2.5	1.2	45–64	
Van der Veen and Bencini	[26]		0.10	2.0	0.8	35–63	
Buzello and Agoston	[30]	Pancuronium	6 mg	2.0	0.7	36–58	2.0
Somogyi *et al.*	[29]		6 mg	1.2	0.7	36–48	
McLeod *et al.*	[28]		4 mg	0.8	0.3–0.4	20–38	
Tassonyi *et al.*	[31]	Pipecuronium	0.07	0.9	0.4	0	33.0
Miller *et al.*	[25]	*d*-Tubocurarine	0.5	8.8	2.9	47–75	3.5
Meijer *et al.*	[32]		0.15	2.9	0.7	17–47	
Hennis and Stanski	[33]		0.10	1.8	0.4	14–35	
Matteo *et al.*	[34]	Metocurine	0.3	6.6	1.1	18–49	10.0
Meijer *et al.*	[32]		0.05	0.6	0.2	7–14	
Weatherley *et al.*	[35]	Atracurium	1.0	16	5	39–82	13.0
Fahey *et al.*	[36]		0.5	4	1	10–35	
Ramzan *et al.*	[37]	Gallamine	2.0	16.8	10	63–70	7.6

these findings is unclear. We did not examine HNMT from other organs or species, and the inhibitory properties of the NMRs may be different for these isozymes. We utilized data from previous pharmacokinetic studies to calculate the concentration of the drugs during distribution and elimination phases [25–40]. The estimated percent of HNMT inhibition was calculated from log [NMR] versus HNMT activity curves assuming SAM and histamine concentration were 1 μM. The calculated decrease in HNMT activity after administration of a single dose of NMRs is shown in Table 3. Each NMR, except pipecuronium, may significantly inhibit HNMT in typical clinical doses. The possible degree of inhibition exerted by NMRs during the distribution phase is not determined by their K_i values for HNMT alone, but rather reflects the ratio of equimolar concentration for neuromuscular blockades to K_i for HNMT inhibition.

Our study further suggests that clinical doses of NMRs could interfere with the HNMT-based radioenzymatic measurement of histamine in plasma. Increasing the concentration of SAM up to 4 μM minimized the inhibition, but could not totally eliminate possible errors in histamine determinations.

In conclusion, the administration of NMRs in intubating doses may potentiate and prolong the clinical signs of histamine liberation caused by other drugs or by themselves.

REFERENCES

1. Clayton DG and Watkins J, Histamine release with vecuronium (letter). *Anaesthesia* **39**: 1143–1144, 1984.
2. Lavery GG, Hewitt AJ and Kenny NT, Possible histamine release after vecuronium (letter). *Anaesthesia* **40**: 389–390, 1985.
3. Conil C. Bornet JL, Jean-Noel M, Conil JM and Brouchet A, Choc anaphylactique au pancuronium et au vecuronium. *Ann Fr Anesth Réanim* **4**: 241–243, 1985.
4. Marshall IG, Agoston S, Booij LHDJ, Durant NN and Foldes FF, Pharmacology of ORG NC 45 compared with other nondepolarizing neuromuscular blocking drugs. *Br J Anaesth* **52**: 11S–18S, 1980.
5. Basta SJ, Savarese JJ, Ali HH, Sunder N, Moss J, Gionfriddo M and Embree P, Vecuronium does not alter serum histamine within the clinical dose range. *Anesthesiology* **59**: A273, 1983.
6. Dobkin AB, Arandia HY and Levy AA, Effects of pancuronium bromide on plasma histamine levels in man. *Anesth Analg* **52**: 772–775, 1973.
7. Booij LHDJ, Krieg N and Crul JF, Intradermal histamine releasing effect caused by ORG-NC 45. A comparison with pancuronium, metocurine and *d*-tubocurarine. *Acta Anaesthesiol Scand* **24**: 393–394, 1980.
8. Beaven MA, Factors regulating availability of histamine at tissue receptors. In: *Pharmacology of Histamine Receptors* (Eds. Ganellin CR and Parsons ME), pp 103–145. Wright PSG, Bristol, 1982.
9. Kusche J, Stahlknecht C-D, Lorenz W, Reichert G and Richter J, Diamine oxidase activity and histamine release in dogs following acute mesenteric artery occlusion. *Agents Actions* **7**: 81–84, 1977.
10. Sattler J, Hesterberg R, Lorenz W, Ennis M, Stahlknecht C-D and Kusche J, Pathophysiological function of diamine oxidase: an evaluation in animal studies using a probabilistic model with several types of causal relationships. In: *Structure and Functions of Amine*

Oxidases (Ed. Mondovi B), pp. 168–178. C.R.C. Press, Boca Raton, FL, 1985.
11. Bowsher RR, Verburg KM and Henry DP, Rat histamine *N*-methyltransferase: quantification, tissue distribution, purification, and immunologic properties. *J Biol Chem* **258**: 12215–12220, 1983.
12. Verburg KM, Bowsher RR and Henry DP, Kinetic analyses of the histamine *N*-methyltransferase reaction as used in the histamine radioenzymatic assay: optimization of assay specificity. *Life Sci* **35**: 241–251, 1984.
13. Futo J, Kupferberg JP, Moss J. Fahey MR, Cannon JE and Miller RD, Vecuronium inhibits histamine *N*-methyltransferase. *Anesthesiology* **69**: 92–96, 1988.
14. Segel IH, *Biochemical Calculations*, 2nd Edn, pp. 246–273. John Wiley, New York, 1976.
15. Moss J and Rosow CE, Histamine release by narcotics and muscle relaxants in man. *Anesthesiology* **59**: 330–339, 1983.
16. Harle DG, Baldo BA and Fischer MM, Inhibition of histamine-*N*-methyltransferase by neuromuscular blocking drugs. *Agents Actions* **17**: 27–31, 1985.
17. Harle DG and Baldo BA, Structural features of potent inhibitors of rat kidney histamine *N*-methyltransferase. *Biochem Pharmacol* **37**: 385–388, 1988.
18. Taylor KM and Snyder SH, Histamine methyltransferase: inhibition and potentiation by antihistamines. *Mol Pharmacol* **8**: 300–310, 1972.
19. Thithapanda A and Cohn VH, Brain histamine *N*-methyltransferase purification, mechanism of action, and inhibition by drugs. *Biochem Pharmacol* **27**: 263–271, 1978.
20. Miller RD and Savarese JJ, Pharmacology of muscle relaxants and their antagonists. In: *Anesthesia*. (Ed. Miller RD) 2nd Edn. Churchill Livingstone, New York, 1986, pp. 889–924.
21. Green H and Erickson RW, Effect of some drugs upon rat brain histamine content. *Int J Neuropharmacol* **3**: 315–320, 1964.
22. Green H and Erickson RW, Effect of some drugs upon the histamine concentration of guinea pig brain. *Arch Int Pharmacodyn Ther* **166**: 121–126, 1967.
23. Taylor KM, Comparison of the antihistaminic effect of burimamide and mepyramine and their effect on the activity of histamine methyltransferase. *Biochem Pharmacol* **22**: 2775–2776, 1973.
24. Netter KJ and Bodenschatz K, Inhibition of histamine-*N*-methylation by some antihistamines. *Biochem Pharmacol* **16**: 1627–1631, 1967.
25. Miller RD, Matteo RS, Benet LZ and Sohn YJ, The pharmacokinetics of *d*-tubocurarine in man with and without renal failure. *J Pharmacol Exp Ther* **202**: 1–7, 1977.
26. Van der Veen F and Bencini A, Pharmacokinetics and pharmacodynamics of ORG NC45 in man. *Br J Anaesth* **52**: 37S–41S, 1980.
27. Fahey MR, Morris RB, Miller RD, Nguyen T-L and Upton RA, Pharmacokinetics of ORG NC 45 (Norcuron) in patients with and without renal failure. *Br J Anaesth* **53**: 1049–1052, 1981.
28. McLeod K, Watson MJ and Rawlins MD, Pharmacokinetics of pancuronium in patients with normal and impaired renal function. *Br J Anaesth* **48**: 341–345, 1976.
29. Somogyi AA, Shanks CA and Triggs EJ, The effect of renal failure on the disposition and neuromuscular blocking action of pancuronium bromide. *Eur J Clin Pharmacol* **12**: 23–29, 1977.
30. Buzello W and Agoston S, Pharmacokinetics of pancuronium in patients with normal and impaired renal function. *Anaesthestist* **27**: 291–297, 1978.
31. Tassonyi E, Szabó G and Vereczkey L, Pharmacokinetics of pipecurium bromide, a new non-depolarizing neuromuscular blocking agent, in humans. *Arzneimittel-Forschung/Drug Res* **31**: 1754–1756, 1981.
32. Meijer DKF, Weitering JG, Vermeer GA and Scaf AHJ, Comparative pharmacokinetics of *d*-tubocurarine and metocurine in man. *Anesthesiology* **51**: 402–407, 1979.
33. Hennis PJ and Stanski DR, Pharmacokinetic and pharmacodynamic factors that govern the clinical use of muscle relaxants. In: *Muscle Relaxants: Basic and Clinical Aspects* (Ed. Katz RL), pp. 179–196. Grune & Stratton, Orlando, 1985.
34. Matteo RS, Brotherton WP, Nishitateno K, Khambatta HJ and Dias J, Pharmacodynamics and pharmacokinetics of metocurine in humans: comparison to *d*-tubocurarine. *Anesthesiology* **57**: 183–190, 1982.
35. Weatherley BC, Williams SG and Neill EAM, Pharmacokinetics, pharmacodynamics and dose–response relationships of atracurium administered i.v. *Br J Anaesth* **55**: 39S–45S, 1983.
36. Fahey MR, Rupp SM, Fischer DM, Miller RD, Sharma M, Canfell C, Castagnoli BA and Hennis PJ, The pharmacokinetics and pharmacodynamics of atracurium in patients with and without renal failure. *Anesthesiology* **61**: 699–702, 1984.
37. Ramzan MI, Triggs EJ and Shanks CA, Pharmacokinetic studies in man with gallamine triethiodide: I. Single and multiple clinical doses. *Eur J Clin Pharmacol* **17**: 135–143, 1980.
38. Tassonyi E, Szabó G and Vimláti L. Pipecuronium bromide (Arduan). *Handbook Exp Pharmacol* **79**: 599–616, 1980.
39. Boros M, Szenohradszky J, Marosi Gy and Tóth I, Comparative clinical study of pipecurium bromide and pancuronium bromide. *Arzneimittel-Forschung/Drug Res* **30**: 389–393, 1980.
40. Sohn YJ, Bencini A, Scaf AHJ, Kersten UW, Gregoretti S and Agoston S, Pharmacokinetics of vecuronium in man. *Anesthesiology* **57**: A256, 1982.