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Role of Histamine in the Hypotensive Action of d-*Tubocurarine in Humans*

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The administration of *d*-tubocurarine (*d*Tc) to animals and humans has been reported to produce hypotension. Experiments in animals suggest that the hypotension is a result of both ganglionic blockade and histamine release. In order to determine the role of histamine release in *d*Tc-induced hypotension in humans, the authors developed a sensitive radioenzymatic assay for plasma histamine and measured plasma histamine following *d*Tc administration (0.25–0.75 mg/kg) to 21 surgical patients. While neither fentanyl (3 μg/kg) nor thiopental (6 mg/kg) produced a significant change in plasma histamine, *d*Tc caused a dose-dependent increase in plasma [dose *d*Tc *vs.* log (plasma histamine), r = 0.62 $P < 0.003$]. The log (plasma histamine) correlated with the *d*Tc-induced hypotension (r = 0.61, $P < 0.005$). The data suggest that histamine release is an important factor in the hypotension accompanying *d*Tc administration in humans. (Key words: Neuromuscular relaxants: *d*-tubocurarine. Histamine. Sympathetic nervous system: ganglia. Blood pressure: drug effects.)

THE ADMINISTRATION OF *d*-TUBOCURARINE (*d*Tc) to animals and humans causes hypotension.[1–8] Experiments in animals suggest that the hypotension is a result of ganglionic blockade.[9,¶] and histamine release.[6,10,11,**] In humans, administration of paralyzing doses of *d*Tc may cause flushing and hypotension, but the role of histamine in these effects has not been demonstrated directly. The purpose of this study was to measure plasma histamine following the administration of *d*Tc to 21 surgical patients during their course of anesthesia using a recently developed radioenzymatic assay for plasma histamine, and to determine if the administration of *d*Tc to humans can cause dose-related elevations in plasma histamine that are well correlated with decreases in blood pressure.

Methods

Twenty-one male patients, ranging in age from 18–56 years (mean = 28 years) were studied. All patients were ASA I or II undergoing peripheral orthopedic procedures. Each patient gave informed consent to an institutionally approved protocol. All patients were premedicated with pentobarbital (3 mg/kg, im) 2 hours prior to the induction of anesthesia. Following the placement of an intravenous cannula in one arm and an additional cannula in the opposite forearm for sample collection, the first sample was drawn. The time from cannula insertion to sampling was not controlled. A study was declared invalid if blood did not flow freely through the sampling catheter. Anesthesia was administered as shown in figure 1. Fentanyl (3 μg/kg) was administered over a 30-s period, a second sample was drawn 2 min later. Thiopental (6 mg/kg) and N_2O/O_2 were given, and a third sample was drawn 4 min later. At this point, *d*Tc, in doses of 0.25–0.75 mg/kg was administered as a bolus. Samples 4, 5, and 6 were drawn two, five, and ten min after injection, respectively. Heart rate and blood pressure were monitored and recorded at each sampling. Patients in the group receiving 0.6 mg/kg of *d*Tc had no samples drawn at point 5.

Blood samples were collected for plasma histamine determination in plastic syringes, then immediately transferred to heparinized tubes (Vacutainer®) and placed on ice. They were centrifuged in a mobile refrigerated centrifuge (2000 rpm × 10 min) within 30 min of collection. The plasma layer was transferred to polypropylene tubes (Falcon 2063®) and stored at −70° C for later analysis.

Plasma histamine was measured utilizing a new assay which is a modification of the single-isotope radioenzymatic assay described by Beaven.[12,13] The biogenic amine was converted to its tritiated methyl derivative using S-(^{3}H) adenosyl-L-methionine (^{3}H-SAMe, 10.0 Ci/mmol, New England Nuclear) as the methyl donor, and histamine-N-methyltransferase (HNMT) isolated

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¶ Alper MH, Flack W: Effects of curare, atropine and halothane on ganglionic transmission in the dog, Abstracts of Scientific Papers. Annual meeting of the American Society of Anesthesiologists, 1969, p. 100.

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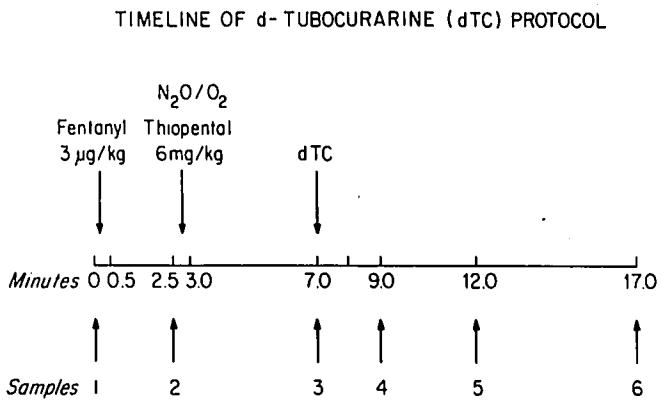

FIG. 1. Time course of drug administration and sample collection.

from rat kidney as the catalyst. Blanks for the assay were incubated without HNMT. Each plasma sample was run in duplicate, with 220 pg exogenous histamine added to a second set of duplicates to serve as internal standards. Each tube (Kimax® glass, 13 × 100 mm, with screw-caps) contained 50 μl plasma, 20 μl 0.1 M phosphate buffer *p*H 7.9 or standard, 10 μl ^{3}H-SAMe (3 μCi), and 10 μl HNMT.

Following incubation at 37° C for 90 min, the reaction was stopped by the addition of 200 μl 0.8 N perchloric acid containing 1 mg/ml 1-methylhistamine (Calbiochem) as a carrier. Two hundred μl 10 N NaOH were added to each tube, followed by 4 ml $CHCl_3$. The methylhistamine was then extracted into the $CHCl_3$ by shaking, centrifugation, and aspiration of the top layer, followed by a wash with 800 μl 3.3 *N* NaOH. After the second aspiration, 2 ml of the organic phase were transferred to a Falcon #2059® polypropylene tube, 17 × 100 mm, and evaporated in a Buchler® vortex-evaporator at room temperature.

The methylhistamine residue was redissolved in 100 μl $CHCl_3$, and each sample was applied to a separate channel of a Whatman® SGLK5DF TLC Plate. The plates were developed in a solvent mixture containing butanol/NH_4OH/ether (80:30:10). After visualizing the methylhistamine band under short-wave UV light, the appropriate segment of each channel (R = 0.78) was scraped into a 20-ml scintillation vial containing 0.5 ml 0.5 N HCl. Ten ml of Liquiscint® (National Diagnostics) was then added, and samples were counted on a Beckman® LS8100. Histamine concentration was determined using the average internal standard of each sample for calculation. Recovery data are presented in table 1.

The advantages of this assay over current methodology are described below. Sensitivity of this assay is 100 pg/ml while omission of the thin layer chromatography step decreases sensitivity sixfold (table 2). Normal values for plasma histamine are 1081 ± 666 pg/ml for six non-premedicated volunteer subjects and 1000 ± 534 pg/ml (n = 21) for control values of our patients. This compares with previously described literature values using less sensitive methodologies.[12–15] Linearity is preserved over a wide range (fig. 2). Intra-assay variation was determined on two different occasions by substituting a control plasma for several samples in an assay. Variability (mean ± SD) was approximately 10 per cent (549 ± 55 pg/ml, n = 6; 484 ± 49 pg/ml, n = 7). Interassay variation was determined over a 4-week period by using control samples taken from a common plasma pool and frozen at −70° C until ready for assaying. The results of these 17 serial measurements were 581 ± 80 pg/ml. No detectable decrease occurred during the storage period.

The data were analyzed by multiple linear regression comparing either drug dose or plasma histamine with the change in systolic blood pressure.

Results

Plasma histamine did not change significantly following the administration of thiopental or fentanyl. Plasma histamine levels 2 min following fentanyl administration were 112 ± 46 per cent (mean ± SD) of control values. Plasma histamine levels showed wider variability (177 ± 262 per cent of control) 4 min after thiopental administration, but were not significantly different from baseline or from fentanyl levels. In part, the large standard deviation in the thiopental group reflected one subject who showed a 1230 per cent increase in plasma histamine following thiopental administration. Exclusion of this subject attenuated the increase to 122 ± 50 per cent baseline levels.

Individual responses of heart rate and systolic blood pressure 2 and 5 min following administration of *d*Tc are presented in figures 3 and 4. There was a great deal of intersubject variability in the hemodynamic response to *d*-tubocurarine administration although there was a tendency for blood pressure to fall and heart rate to rise. Plasma histamine response was also highly variable (data presented in fig. 5). Administra-

TABLE 1. Recovery of Histamine Added to Human Plasma

Amount of Histamine Added* (pg)	Histamine Found† (pg)	Histamine Recovered (per cent)
55	65 ± 32	118
110	99 ± 24	90
550	547 ± 39	99
1100	1101 ± 18	100

* Histamine was added to a 50-μl plasma sample.
† Values are means ± SD (n = 6).

tion of *d*-tubocurarine at 0.25 mg/kg produced an increase in only two patients, while all patients except one receiving greater than 0.25 mg/kg, demonstrated at least twofold increases in plasma histamine. A comparison of histamine release at 0.25 mg/kg and 0.5 mg/kg is given in figure 6. The large standard deviation following administration of the 0.25 mg/kg dose (fig. 6) reflected the individual variability seen in figure 5. Values obtained 5 min following administration of *d*-tubocurarine were not significantly different from baseline values. The change in systolic blood pressure (measured as the ratio of systolic blood pressure 2 min after administration of *d*-tubocurarine to the systolic blood pressure obtained just prior to drug administration) was closely related to the absolute level of plasma histamine (fig. 7). Multiple linear regression analysis utilizing drug dose or plasma histamine *vs.* the change in systolic blood pressure revealed that drug dose correlated significantly with log plasma histamine ($r = 0.62$, $P < 0.003$), and log plasma histamine correlated inversely with the systolic blood pressure ratio ($r = 0.61$, $P < 0.005$). Interestingly, the direct relationship between drug dose and hemodynamic changes was weaker ($r = -0.44$, $P < 0.05$).

Discussion

Hypotension following the administration of *d*-tubocurarine has been described in experimental animals and humans.[1–8] The mechanism for this hypotension has been postulated to be histamine release[6,10,11] or partial ganglionic blockade.[9] The evidence for histamine release in humans has been indirect, *i.e.*, elicitation of the Triple Response of Lewis, or delayed depressor response.

Initial experiments in animals failed to demonstrate significant changes in blood histamine levels following the administration of *d*-tubocurarine.[11] Subsequent experiments in dogs and cats using improved fluorometric methodology established that *d*-tubocurarine caused a dose-dependent fall in mean arterial pressure accompanied by an increase in whole blood histamine.[7] While whole blood histamine includes plasma histamine, it is also a reflection of basophil number and is thus a less valid measure of histamine release from tissue stores.

TABLE 2. Effect of Thin Layer Chromotography (TLC) on Assay Sensitivity

Amount of Histamine (pg)	Before TLC			After TLC		
	CPM	CPM/pg	Sample/ Blank	CPM	CPM/pg	Sample/ Blank
0	1185	—	—	60	—	—
55	2557	46	2.16	825	15	13.75
110	3730	34	3.15	1572	14	26.20
550	16764	30	14.15	6920	13	115.33
1100	32847	30	27.72	13738	12	228.97

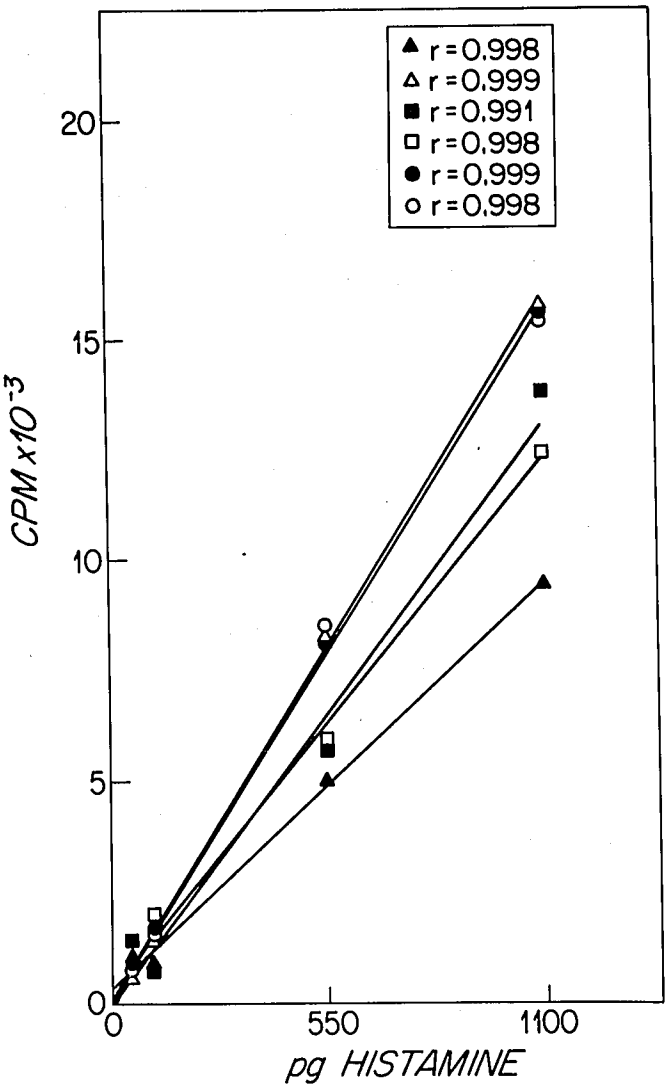

FIG. 2. Validation of the linearity of histamine assay. Exogenous histamine (0–1100 pg) was added to plasma samples (50 μl) obtained from six normal subjects. Regression coefficients for these dose-response curves are indicated in the figure.

Plasma histamine levels in humans which normally are 600–1,000 pg/ml,[12–15] have been measured using radioenzymatic methodology, but few experiments have documented dose-dependent increases in plasma histamine in response to drug administration. It has long been suspected on the basis of clinical observations that hemodynamically significant drug-induced histamine release does occur in humans, but the extent to which such release was related to hypotension has only recently been examined.[16–18] Lorenz and Doenicke reported that the intravenous administration of histamine to normal men caused increases in

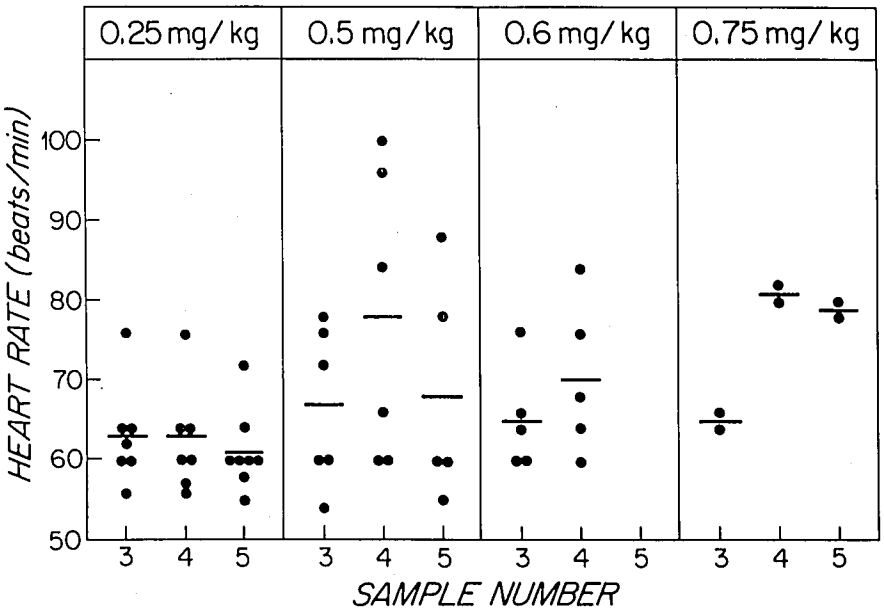

FIG. 3. Heart rate following administration of doses of *d*Tc to study patients. Horizontal bars indicate mean values. Heart rate was not recorded in one patient in the 0.5 mg/kg group (event 5) and one patient in the 0.6 mg/kg group (events 3 and 4).

pulse rate in 75 per cent of the subjects examined, but no hypotension was apparent.[17] In another series of patients who manifested anaphylactoid reactions to propanidid and dextran infusions, marked hypotension was present along with significant increases in plasma histamine levels.[16,18] The use of H_1 and H_2 receptor blockers in these susceptible individuals could prevent the hemodynamic effect.[16] Other studies by Lorenz and colleagues demonstrated threefold increases in plasma histamine levels following the administration of thiopental, but these occurred without significant changes in blood pressure in normal men.[18]

The lack of a sensitive reliable assay for plasma his-

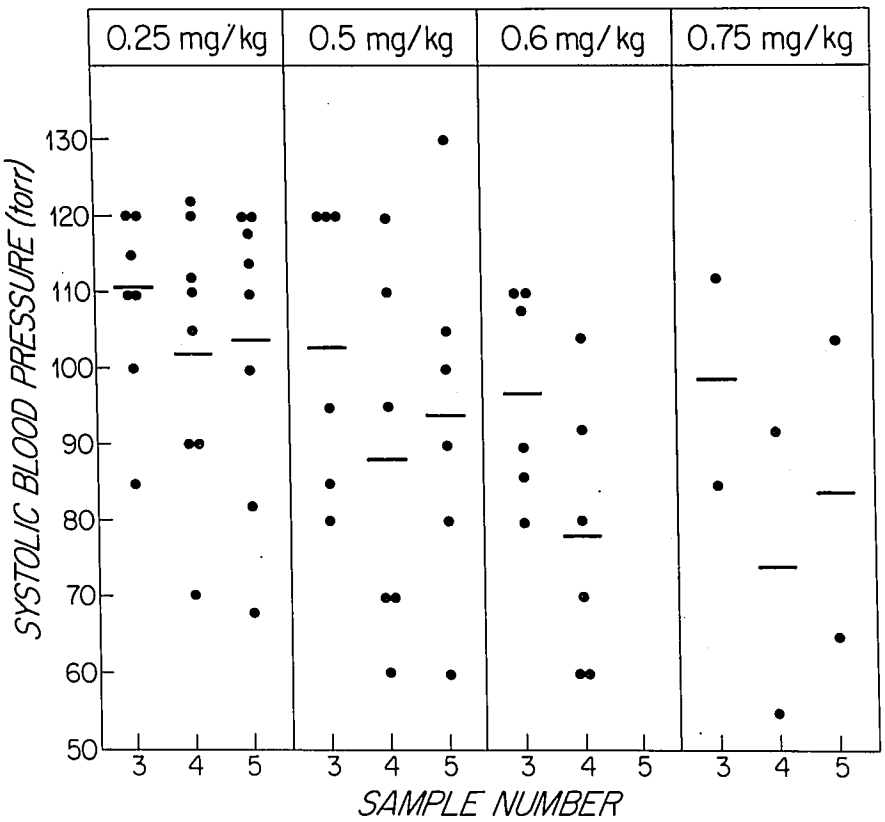

FIG. 4. Systolic blood pressure following administration of doses of *d*Tc to study patients. Horizontal bars indicate mean values.

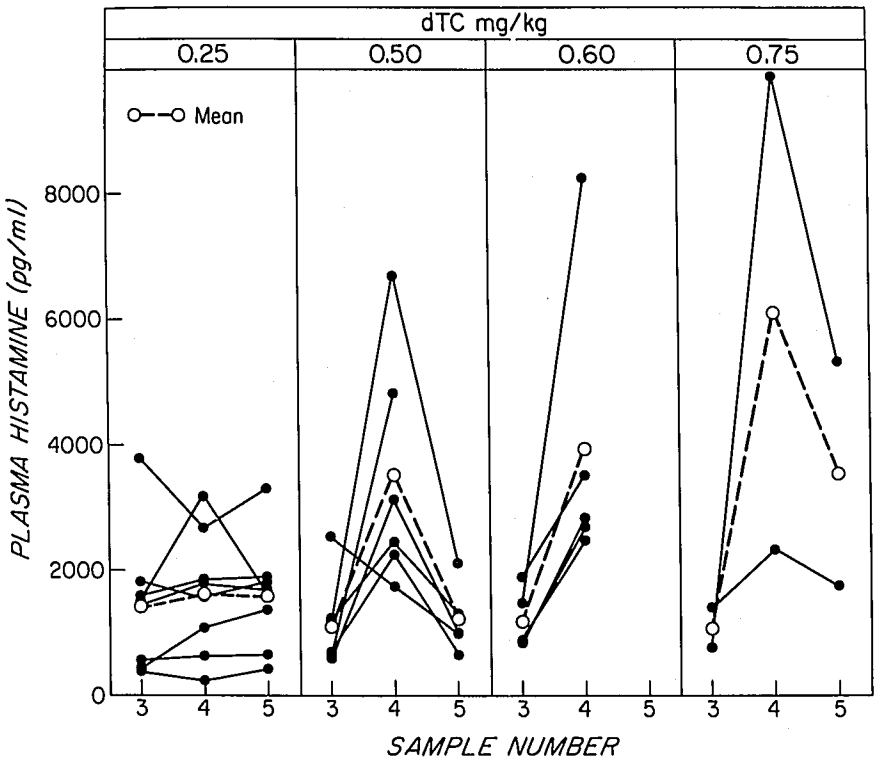

FIG. 5. Plasma histamine following administration of indicated doses of *d*Tc to study patients. Dotted lines connect mean values. Plasma histamine was not measured in one patient in the 0.5 mg/kg group (event 5) and one patient in the 0.6 mg/kg group (events 3 and 4).

tamine has made it difficult to document its role in drug-induced cardiovascular changes. The development of a radioenzymatic assay for histamine[12,19–21] and its recent improvement by the discovery of renal histamine-N-methyl transferase[13] greatly enhanced our ability to detect plasma histamine in clinically important situations. We have further refined the radioenzymatic assay by utilizing high specific activity s-adenosylmethionine as the methyl donor and separating off possible contaminants with thin layer chromatography. The linearity of the assay is preserved over a wide range of values (see fig. 2). Specificity is conferred by the enzyme, so it is reasonable to expect that those compounds which compete for the enzyme would exert competitive or noncompetitive inhibition.[22] Since our internal standards remained unchanged between samples in the same patient, there was apparently no such interference in our study. It should also be noted that plasma from different individuals exerted varying levels of internal inhibition (noted as the difference in slopes of fig. 2), but linearity was preserved within the same individual. The extent to which such internal inhibition occurs will determine the sensitivity of the assay for each individual, but in none of our subjects was the baseline histamine level undetectable. The sensitivity of the method for human plasma histamine is 100 pg/ml or better, well below the normal range. The changes seen during the administration of *d*-tubocurarine could not be accounted for by inter- or intra-assay variation.

Our data demonstrate that the administration of *d*-tubocurarine to normal patients causes a release of plasma histamine which is manifested as an increase in plasma levels. While *d*-tubocurarine could alter histamine metabolism, the lack of inhibition in the internal standard and the rapidity of the increase suggests this is not the case, and that the elevation in plasma levels reflects release. Even at the lowest dose of curare (0.25 mg/kg), two of the six patients examined showed greater than 50 per cent increases in plasma levels within 2 min of injection. At higher doses (0.5–0.6 mg/kg) histamine increased significantly in all but one of the patients examined. Both patients who received 0.75 mg/kg of curare as a bolus showed significant increases in plasma histamine levels. The extent of hypotension observed precluded investigation of other patients at this dosage level.

It is of note that within 5 min of administration of *d*-tubocurarine, plasma histamine returned to control values. This suggests a relatively short half-life for plasma histamine. Since no intermediate values were obtained, the exact half-life cannot be computed. While thiopental has been reported to elevate plasma histamine, we did not detect an increase 4 min after its administration.[16,18] We chose this time interval in order to minimize the possibility of thiopental-induced histamine release. Since were were unable to detect a significant increase over baseline following the administration of 0.25 mg/kg *d*-tubocurarine, we believe it unlikely that thiopental was contributing to the

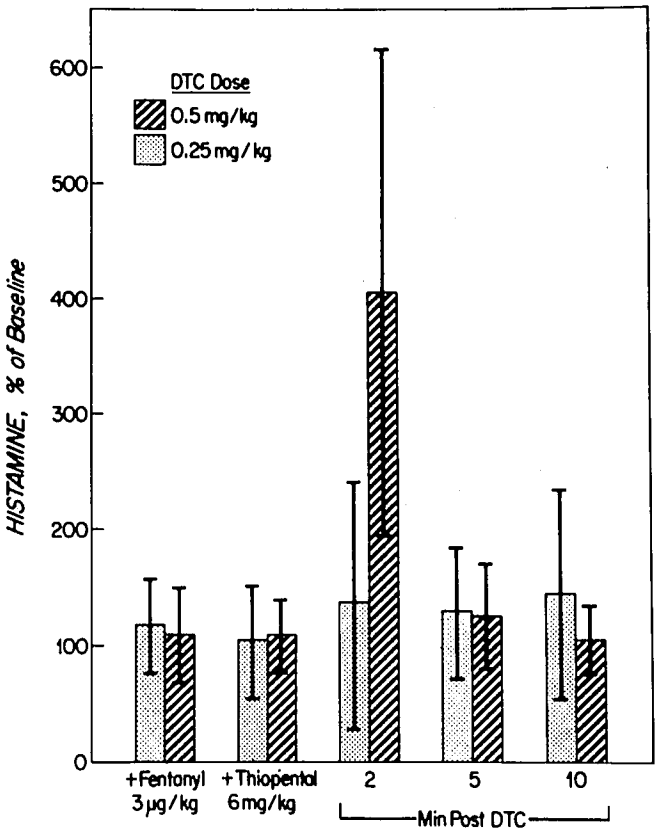

FIG. 6. Changes in plasma histamine concentration following administration of *d*-tubocurarine at doses of 0.25 mg/kg (n = 8) and 0.5 mg/kg (n = 6). Changes are expressed as percent of baseline histamine levels ± SD. The increases at 2 min after 0.5 mg/kg are significantly different ($P < 0.005$) from both the histamine concentration immediately before the drug was given and from the increase observed 2 min after a dose of 0.25 mg/kg *d*Tc. Plasma histamine in the 0.25 mg/kg dose did not significantly change from baseline following fentanyl, thiopental, or *d*-tubocurarine administration.

increase in plasma histamine we observed at higher *d*Tc dosage.

Our observations were made on venous plasma histamine. Recent experience with cardiac surgical patients suggests that venous levels may be somewhat higher than arterial levels, presumably because of the large store of histamine within the skin and mesentery.†† The measured plasma levels reflect histamine release from tissues, but presumably do not account for the large amounts of histamine metabolized or taken up within the tissue bed. Similar observations have been made regarding norepinephrine release.[23] Thus, it is not surprising that plasma levels achieved with infusion of exogenous histamine can be identical with levels achieved by drug administration, but still may not precipitate hemodynamic changes of the same magnitude.[24]

While ganglionic blockade secondary to the administration of *d*-tubocurarine has been demonstrated to occur in various species,[9,23] our data suggest that the release of histamine, as measured by plasma histamine levels, explains a significant amount of the hypotension that occurs with this drug. Measurement of ganglionic blockade is difficult in the clinical setting, so it is virtually impossible to establish how important a role it plays in normal anesthetized humans. Savarese, using the chloralose-anesthetized cat, established that ganglionic blockade induced by *d*-tubocurarine occurred at 1.35 mg/kg, while the delayed depressor response was transient and occurred at 0.40 mg/kg.[25] Data accumulated during these experiments suggested that the duration of ganglionic blockade secondary to *d*-tubocurarine administration was similar to the duration of the neuromuscular blockade.‡‡ In our patients, the relatively prompt return of blood pressure to normal levels follows the rapid return to control plasma histamine levels, despite continued neuromuscular blockade. The multiple linear regression analysis showed a significant relationship between

†† Moss J: unpublished observations.

‡‡ Savarese JJ: unpublished observations.

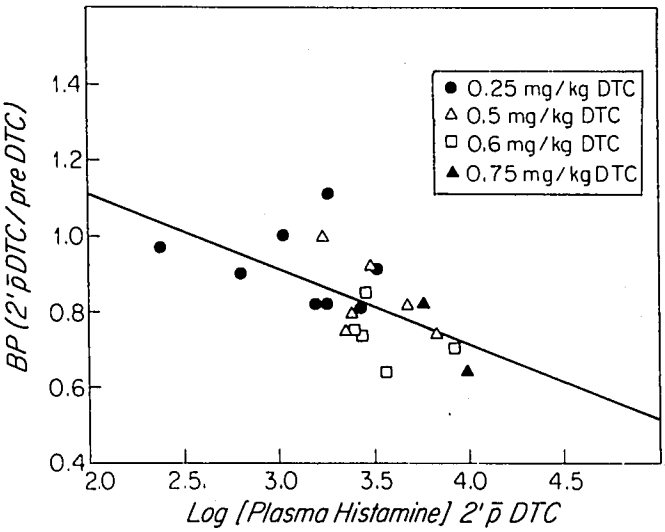

FIG. 7. Relationship between the decrease in blood pressure and plasma histamine concentration 2 min after the administration of indicated doses of *d*-tubocurarine in 21 patients. The regression line for these data points is $y = -0.2x - 1.5$, with a correlation coefficient of $r = -0.61$ ($P < 0.005$). The equation relating the three variables was:

$$\frac{\text{Systolic blood pressure 2 min after } d\text{Tc}}{\text{Systolic blood pressure before } d\text{Tc}} = 1.37 - 0.27 \text{ (dose } d\text{Tc)} - 0.12 \text{ Log histamine}$$

the dose of *d*-tubocurarine and the log transform of plasma histamine, and between log plasma histamine and the blood pressure change. A much less powerful correlation between the drug dose and hypotension was obtained. This supports histamine as an important mediator of the hypotension. Thus, on the basis of the relationship between hypotension and plasma histamine and the time course, ganglionic blockade would seem to be a less probable cause for the observed hypotension.

Hypotension induced by *d*-tubocurarine is temporally and quantitatively related to the absolute levels of plasma histamine. Measurement of histamine levels should establish the role of this biogenic amine in the hemodynamic effects of a variety of clinically useful anesthetic drugs.

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