Different Patterns of Mast Cell Activation by Muscle Relaxants in Human Skin

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Background: Activation of mast cells and systemic release of histamine are major side effects of intravenously administered muscle relaxants. In the current study, dermal microdialysis was used for the investigation of mast cell activation by muscle relaxants. Dermal microdialysis enabled simultaneous assessment of mediator release, vascular reactions, and sensory effects induced by intradermal application of muscle relaxants without systemic side effects.

Methods: Succinylcholine, the isoquinolines cisatracurium, atracurium, and mivacurium, and the steroids pancuronium, vecuronium, rocuronium, and rapacuronium were tested in human volunteers (n = 6 each). After intradermal insertion of microdialysis capillaries (0.4 mm diameter, cutoff 3,000 kd) and a 60-min equilibration period, the muscle relaxants were delivered *via* the capillaries for 30 min, followed by a 30-min washout period. Dialysate was sampled at 15-min intervals, and histamine, mast cell tryptase, and protein extravasation were determined. Changes in skin blood flow were measured using a laser Doppler imager. Potency and efficacy were derived from nonlinear fittings of the dose–response curves.

Results: For succinylcholine and the isoquinolines, dose–response curves for the vascular and sensory effects paralleled the histamine and tryptase release. In contrast, aminosteroids evoked a rapid histamine release that was accompanied by a delayed increase in tryptase.

Conclusions: Dermal microdialysis has been successfully used to simultaneously assess mediator release, vascular reactions, and sensory effects. The different pattern of tryptase release by isoquinolines and aminosteroids suggests different mechanisms of mast cell activation.

ANAPHYLACTIC or anaphylactoid reactions have been reported frequently as adverse reactions caused by muscle relaxants.[1,2] Mast cell degranulation and the release of vasoactive mediators is regarded as the main mechanism. Muscle relaxants may bind to specific immunoglobulin E antibodies on mast cells of sensitized patients, but more frequently nonimmunologic mechanisms account for the activation of mast cells.[1,3,4] On stimulation, mast cells release a variety of preformed and newly synthesized inflammatory mediators, including hista-mine, interleukins, and tumor necrosis factor α. In addition, proteinases (tryptase, chymase, and carboxypeptidase) and newly generated lipid mediators such as prostaglandin D_2, leukotriene C_4, and platelet-activating factor are liberated.[5-7] Although the intracellular signaling of immunologic and nonimmunologic activation of mast cells differs considerably (tyrosine kinases *vs.* activation of G proteins and phospholipase C and A_2[8-10]), only minor differences in mediator release were reported concerning prostaglandin D_2 and leukotriene C_4, which are released only on activation *via* immunoglobulin E.[9] Histamine has been regarded as the major mediator for the anaphylactic or anaphylactoid reactions. However, because of its short half-life in plasma, it has been suggested that tryptase is a better measure.[3] In addition, recent work suggested that tryptase might not only indicate mast cell activation, but also participates in the inflammatory process. It activates proteinase activated receptor 2 (PAR-2), which has been located on various tissues, including nociceptors,[11,12] and even on mast cells themselves.[13] By activating these receptors, tryptase induced a local inflammation indicated by enhanced protein extravasation, vasodilatation, and leukocyte infiltration.[14-16]

The activation of mast cells by muscle relaxants has been tested previously using *in vitro* and *in vivo* settings.[17-19] However, in the *in vitro* situation, the interaction of mast cells with vessels and nerves cannot be investigated. On the other hand, after intradermal injection, released mediators cannot be quantified. We therefore used dermal microdialysis to simultaneously deliver the relaxants to the skin and measure locally released mediators. In combination with scanning laser Doppler imaging, we were able to simultaneously determine time courses of histamine and tryptase release, protein extravasation, local vasodilation, and axon reflex flare during *in vivo* conditions.

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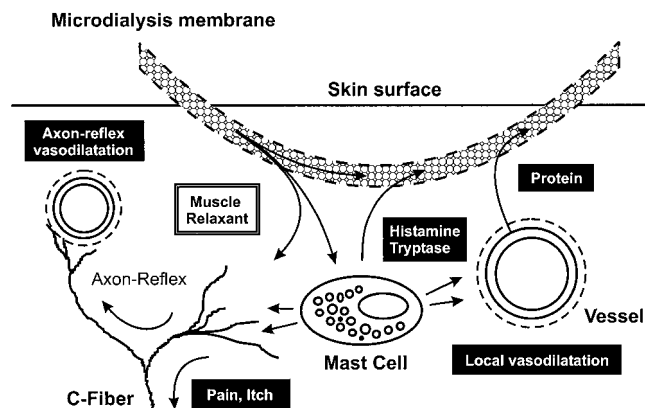
Materials and Methods

Forty-eight healthy subjects (25 women and 23 men; mean age, 26 yr; range, 20–37 yr) participated in this randomized, double-blind study. They were randomly designated to one of eight study groups, each receiving one muscle relaxant in different concentrations. None had previously suffered from a hypersensitivity to drugs, especially muscle relaxants, or was taking medication that could interfere with itch or pain sensations and flare response (*i.e.*, analgesics, antihistamines, cromoglycate,

Fig. 1. Schematic illustration of the experimental setup. The muscle relaxant was delivered by diffusion *via* plasmapheresis hollow fibers inserted intracutaneously, causing mast cell degranulation. Mediator release, vascular reactions, and sensory effects were determined.

calcium, or sodium channel blockers). Each subject gave informed consent to take part in the study, and the experimental protocol was approved by the Ethics Committee of the Medical Faculty of the University of Erlangen-Nuremberg.

Experimental Protocol

Stock Solutions. The depolarizing muscle relaxant succinylcholine chloride (10 mg/ml; Nycomed, Ismaning, Germany), the nondepolarizing benzylisoquinolinium compounds cisatracurium (2 mg/ml; GlaxoWellcome, Hamburg, Germany), atracurium (10 mg/ml, GlaxoWellcome), and mivacurium (2 mg/ml, GlaxoWellcome), as well as the nondepolarizing aminosteroid muscle relaxants pancuronium bromide (2 mg/ml; Organon Teknika, Eppelheim, Germany), vecuronium bromide (1 mg/ml, Organon Teknika), rocuronium bromide (10 mg/ml, Organon Teknika), and rapacuronium bromide (20 mg/ml; Organon Inc., West Orange, NJ) were used.

Microdialysis. In each subject, six to seven single plasmapheresis hollow fibers (diameter, 0.4 mm; cutoff, 3,000 kd; Dermal Dialysis, Erlangen, Germany) were inserted intracutaneously at a length of 1.5 cm *via* a 25-gauge cannula in the volar forearm. All fibers were orientated transversally to the axis of the forearm with at least 3 cm distance. The hollow fibers were perfused with Ringer's solution (Ringerlösung, Bad Homburg, Germany) using a microdialysis pump (Pump 22, Harvard Apparatus, South Natick, MA) at a flow rate of 4 μl/min (experimental setup; fig. 1). Tygon® tubes (Cole-Parmer Instrument Company, Vernon Hills, IL) were used to connect the hollow fibers with the syringes (ID = 0.38 mm, length = 10 cm). Insertion depth was measured by ultrasound (Dermascan C; Cortex Technologies, Hadsund, Denmark) to be 0.7 mm on average (range, 0.5–0.9 mm). After skin passage, the membranes were inserted in glass capillaries for collecting the dialy-

sate. To minimize outflow resistance, the capillaries were tilted at an angle of 5°. The length of microdialysis fibers being exposed to air was less than 3 mm on the inflow and outflow sites. Dialysate samples were taken every 15 min for 120 min and frozen at −20°C in polyethylene cups until analysis.

After a baseline of 60 min, five to six fibers were perfused with different concentrations of one muscle relaxant (one concentration per fiber) for 30 min (stimulation period); one fiber perfusion with an acidified solution (NaCl–HCl, pH adjusted to 4) served as control. The stimulation period was followed by a 30-min washout period. The application of the drugs did not involve an additional trauma (*e.g.*, intracutaneous injection), but was driven only by diffusion from the dialysis fibers, thereby limiting the absolute amount of applied drug.

Sample Analysis. Each dialysate sample (60 μl) was analyzed for total protein (5 μl), histamine (20 μl), and tryptase (30 μl) content. Total protein content was measured photometrically (MRX reader; Dynatech, Denkendorf, Germany) using Coomassie blue dye for the analysis and bovine serum albumin as a standard.[20] Histamine was analyzed using a fiber-based spectrofluorometric assay. The principle and analytical aspects of this assay in different types of biologic samples, *e.g.*, whole blood, isolated mast cells, and nasal lavage fluid, have been described in detail.[21] The limit of detection of this assay is 5 ng/ml; the fluorescence output is linear up to approximately 1,000 ng/ml. Tryptase was analyzed by radioimmunoassay according to the manufacturer's instructions (UniCAP 100; Pharmacia & Upjohn, Freiburg, Germany). The assay has a sensitivity of 1 ng/ml tryptase and a linear range up to 200 ng/ml.

Rating. During the first minute after onset of stimulation, the subjects were asked to rate the maximum itch or pain sensation on numeric rating scales (NRS) separately for each stimulation site. The end points of the scale were defined as "no itch–pain" (numeric rating scale = 0) and "maximum itch–pain" (numeric rating scale = 10).

Flare Analysis. Superficial blood flow of the forearm was measured repeatedly by laser Doppler imager (LDI; Moor Instruments Ltd., Devon, United Kingdom). For this purpose, an area of 20 × 10 cm around the injection sites were scanned with a resolution of 16,380 pixels. They were recorded for further processing with dedicated software (MoorLDI Version 3.0, Moor Instruments Ltd.). The mean flux was determined separately in an area of 1.5 × 0.4 cm around the respective fiber to determine the local vasodilatation and in a rectangular area of the same size at a distance of 1 cm from the fiber to determine the axon-reflex vasodilatation. The flare area was calculated from all pixels around the fiber in which flux values after stimulation with the muscle relaxant exceeded the 99% percentile of the baseline distribution measured immediately before the stimulation.

In Vivo Delivery

The *in vivo* delivery of the muscle relaxants was determined in eight subjects. The delivery was calculated as the relative difference between the amount of muscle relaxant in the perfusate and the dialysate (percent decline in concentration). Succinylcholine was analyzed using high-performance liquid chromatography and fluorescence detection. The benzylisoquinolines and aminosteroids were analyzed by reversed-phase liquid chromatography electrospray ionization mass spectrometry.[22] All drugs were stabilized to avoid hydrolysis: 10 μl echothiopate iodide (50 mg/ml, mivacurium) or 4 ml H_2SO_4 (0.015 M atracurium, cisatracurium) were added immediately to 1 ml of the diluted benzylisoquinoline samples, and 200 μl NaH_2PO_4 (1 M) was added to 2 ml of the diluted aminosteroid samples. The samples were kept frozen ($-20°C$) until analysis. The samples were diluted in 50 mM ammonium formate buffer (pH 3) before analysis and were injected without previous extraction. The mobile phase consisted of 50 mM ammonium formate buffer (pH 3) and methanol in different ratios, depending on the substance to be analyzed. The limit of detection was 2 ng/ml for the benzylisoquinolines and 10 ng/ml for the aminosteroids (5 ng/ml rocuronium bromide). The calibration curve was linear up to 2,500 ng/ml (benzylisoquinolines) and 3,000 ng/ml (aminosteroids), respectively. The within- and between-day precision was less than 2% for all substances.

Statistical Analysis

Results are expressed as mean $\pm$ SD. Potencies are given as concentration required for 50% of the maximum effect (ED_{50}). For each subject, ED_{50} values and efficacy (maximum effect) of the respective muscle relaxant were obtained by nonlinear regression fitting to the logistic function:

$$y = y_{max} + (y_{min} - y_{max})/(1 + (x/x_{50})^n),$$

where n is the Hill coefficient, y_{max} represents efficacy, and x_{50} is the concentration required for ED_{50}. A Hill coefficient greater than 1 indicates cooperativity, and higher values are combined with steeper shape of the sigmoidal dose–response curve. For these calculations, Origin software (Microcal, Northampton, MA) was used. Data were compared using one-way analysis of variance followed by Newman-Keuls *post hoc* tests. Correlations were described using the Pearson correlation coefficient (r). Significance levels throughout the study were $P < 0.05$. The Statistica software package (Statsoft, Tulsa, OK) was used for statistical analysis.

Results

In Vivo Delivery

During stimulation, *in vivo* deliveries were 30–35% for succinylcholine and the benzylisoquinolines and 25–30%

for the aminosteroids (n = 4 each). We did not detect any of the muscle relaxants in the samples 15 min after the end of the stimulation. Adverse systemic effects were not observed.

Dose Dependency of Effects

Intradermal stimulation with each muscle relaxant led to dose-dependent increases of intradermal histamine and tryptase, causing protein extravasation and vasodilatation as well as itch and pain sensations. Stimulation with Ringer's solution or the acidified solution did not induce histamine and tryptase release.

As shown in figure 2, mivacurium and rapacuronium provoke dose-dependent effects on histamine and tryptase release, on protein extravasation, as well as local and axon-reflex vasodilatation. Kinetics of histamine release did not differ between the two relaxants. However, the time course of tryptase release and of vascular reactions matched the time course of histamine release only for mivacurium. In contrast, rapacuronium induced a delayed and protracted release of tryptase.

Potency and efficacy were obtained for each muscle relaxant with the exception of vecuronium and, in part, for pancuronium and succinylcholine (table 1). Within the tested range, increasing concentrations of vecuronium did not reach a maximum for mediator release as well as local and axon-reflex vasodilatation. After stimulation with increasing concentrations of pancuronium and succinylcholine, no maximum for local and axon-reflex vasodilatation was observed.

Rating

During the first minute of stimulation, each muscle relaxant showed dose-dependent effects on itch or pain sensations (fig. 3). Newman-Keul *post hoc* tests revealed the highest itch ratings in subjects receiving rapacuronium ($P < 0.01$). Vecuronium and rocuronium elicited significantly less itch sensations when compared with all other muscle relaxants ($P < 0.05$). Itch ratings were positively correlated with histamine and protein release (r = 0.66 and r = 0.60, respectively; $P < 0.001$), as well as axon-reflex erythema (r = 0.70; $P < 0.001$).

The highest pain ratings were determined in subjects stimulated with rocuronium ($P < 0.01$). Succinylcholine, atracurium, mivacurium, and rapacuronium elicited significantly less pain sensations than the other muscle relaxants ($P < 0.05$). Only weak positive correlations were found for pain ratings and mediator release or vasodilatation (r < 0.3; $P < 0.01$).

Mediator Analysis

The muscle relaxants dose-dependently induced histamine and tryptase release as well as protein extravasation with different potencies ($P < 0.01$; fig. 4). However, individual muscle relaxants showed equal release potencies for the respective mediators (table 1). ED_{50} values
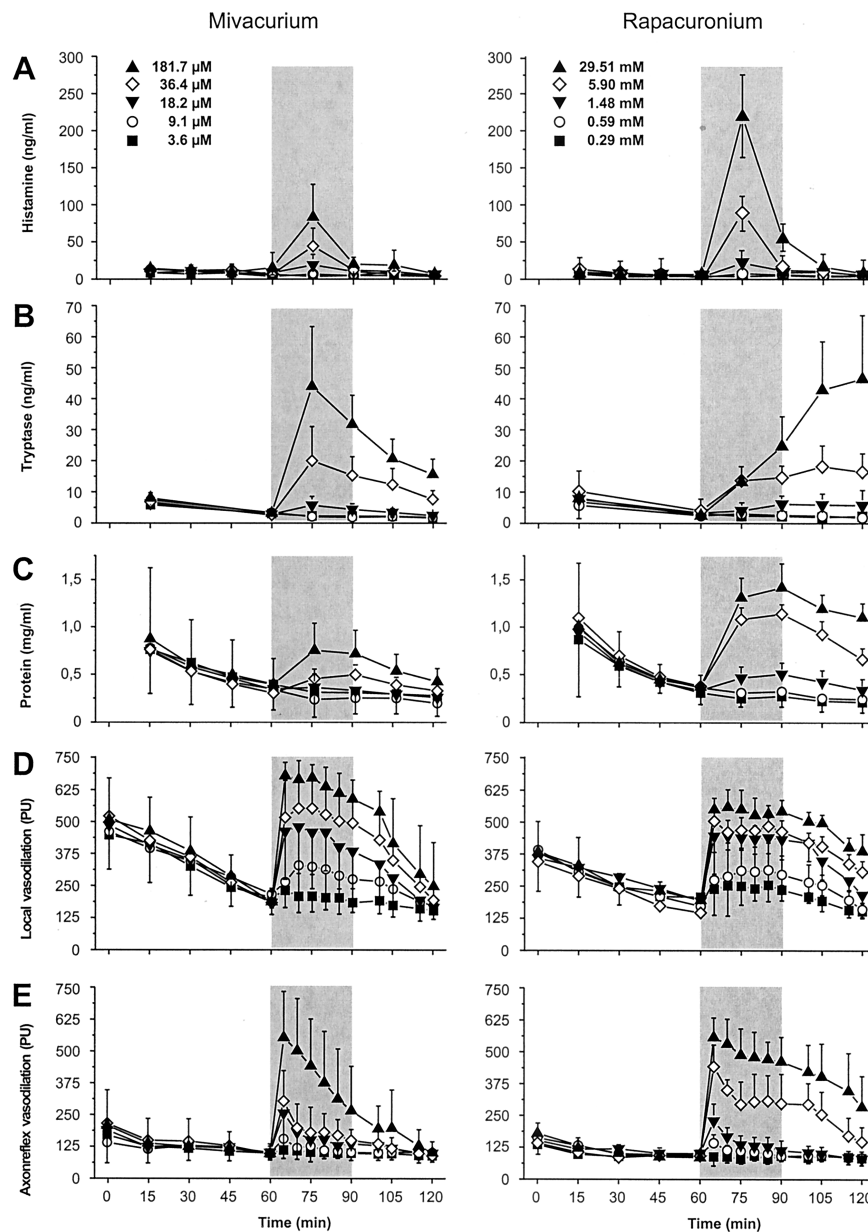
Fig. 2. Time courses of histamine and mast cell tryptase release (*A* and *B*), protein extravasation (*C*), and local (*D*) as well as axon-reflex vasodilatation (*E*) after intradermal stimulation (shaded areas) with different concentrations of mivacurium (*left*) and rapacuronium (*right*). Data are expressed as mean ± SD.

for succinylcholine were significantly higher than those of rapacuronium and rocuronium, whereas ED_{50} values of the other muscle relaxants were significantly smaller ($P < 0.05$, respectively, by analysis of variance, Newman-Keul *post hoc* tests).

Efficacy of atracurium and rapacuronium to release histamine was significantly higher than with each other muscle relaxant ($P < 0.05$ and $P < 0.01$, respectively; table 1). In contrast, efficacy for tryptase release during stimulation was higher for benzylisoquinolines than for aminosteroids ($P < 0.05$). No differences were found in efficacy of the muscle relaxants to extravasate protein (nonsignificant).

Different patterns of mediator release during stimulation with each muscle relaxant led to distinct histamine/tryptase ratios (table 2). Aminosteroids showed a fivefold higher hista-

mine/tryptase ratio compared with benzylquinolines and succinylcholine during stimulation ($P < 0.01$). In contrast, during the washout period, the histamine/tryptase ratio was significantly smaller with the aminosteroids when compared with benzylisoquinolines and succinylcholine ($P < 0.05$).

Flare Analysis

During stimulation with muscle relaxants, dose-dependent increases in local and axon-reflex vasodilatation were determined, causing extended flare areas (fig. 5). In parallel with the mediator release, muscle relaxants induced flare responses with different potencies ($P < 0.01$; table 1). However, efficacy for local and axon-reflex vasodilatation as well as flare areas was not significantly different (table 1).

Table 1. Potencies (ED$_{50}$s) and Efficacies for Intradermal Mediator Release

	Succinylcholine	Cisatracurium	Atracurium	Mivacurium	Pancuronium	Rocuronium	Rapacuronium
Histamine							
Stimulation							
ED$_{50}$ (mM)	23.64 ± 6.39	0.36 ± 0.20	0.37 ± 0.04	0.04 ± 0.02	0.88 ± 0.32	2.99 ± 0.74	7.94 ± 2.48
Efficacy (ng/ml)	35.1 ± 20.1	40.5 ± 29.0	101.6 ± 27.7	53.1 ± 23.4	39.6 ± 19.0	32.9 ± 20.2	143.1 ± 43.2
Wash-out							
ED$_{50}$ (mM)	23.13 ± 3.21	0.29 ± 0.11	0.26 ± 0.11	0.04 ± 0.02	0.73 ± 0.32	2.35 ± 0.99	7.36 ± 2.22
Efficacy (ng/ml)	9.1 ± 2.3	12.9 ± 4.2	33.6 ± 26.6	27.4 ± 16.4	23.3 ± 14.0	10.9 ± 7.6	14.0 ± 5.7
Tryptase							
Stimulation							
ED$_{50}$ (mM)	36.70 ± 6.40	0.42 ± 0.06	0.47 ± 0.13	0.05 ± 0.03	0.76 ± 0.25	2.78 ± 1.13	5.35 ± 1.51
Efficacy (ng/ml)	31.1 ± 12.6	50.2 ± 37.3	73.0 ± 47.8	46.3 ± 39.5	5.4 ± 1.7	5.2 ± 1.8	25.4 ± 9.9
Wash-out							
ED$_{50}$ (mM)	34.37 ± 6.25	0.47 ± 0.16	0.41 ± 0.15	0.05 ± 0.02	0.81 ± 0.34	3.35 ± 1.61	5.27 ± 1.60
Efficacy (ng/ml)	8.7 ± 4.8	21.4 ± 14.4	30.7 ± 18.0	20.7 ± 14.3	17.1 ± 8.5	22.0 ± 10.1	48.3 ± 27.0
Protein							
Stimulation							
ED$_{50}$ (mM)	27.59 ± 9.63	0.32 ± 0.10	0.33 ± 0.15	0.04 ± 0.01	0.69 ± 0.14	3.58 ± 0.94	3.72 ± 1.10
Efficacy (mg/ml)	0.63 ± 0.22	0.92 ± 0.47	1.39 ± 0.45	0.93 ± 0.22	1.15 ± 0.66	1.23 ± 0.40	1.45 ± 0.28
Wash-out							
ED$_{50}$ (mM)	22.35 ± 12.71	0.33 ± 0.15	0.34 ± 0.18	0.04 ± 0.02	0.68 ± 0.17	3.05 ± 1.42	3.84 ± 1.35
Efficacy (mg/ml)	0.47 ± 0.24	0.79 ± 0.34	0.86 ± 0.35	0.48 ± 0.14	1.17 ± 0.72	0.96 ± 0.25	1.24 ± 0.27
Local vasodilatation							
ED$_{50}$ (mM)		0.23 ± 0.06	0.21 ± 0.09	0.03 ± 0.01		2.76 ± 1.31	4.72 ± 0.89
Efficacy (PU)		4,014 ± 909	3,801 ± 675	3,950 ± 322		3,644 ± 658	4,212 ± 691
Axon-reflex vasodilatation							
ED$_{50}$ (mM)		0.28 ± 0.11	0.31 ± 0.05	0.05 ± 0.02		3.62 ± 1.69	3.69 ± 1.65
Efficacy (PU)		2,919 ± 948	2,965 ± 637	3,104 ± 1,305		2,004 ± 266	2,978 ± 1,191
Flare area							
ED$_{50}$ (mM)		0.27 ± 0.13	0.29 ± 0.11	0.05 ± 0.02		3.40 ± 1.78	4.35 ± 1.14
Efficacy (cm^2)		14.31 ± 3.43	11.30 ± 2.64	16.58 ± 6.55		13.30 ± 5.88	18.05 ± 4.67

Data are mean ± SD.

ED$_{50}$ = concentration required for half maximum effect; efficacy = maximum effect.

Histamine-releasing Activity

Table 3 gives the comparative histamine-releasing potency, taking into account the varying dosage required for muscle relaxation. Concentrations required to produce half maximum histamine release in our setting were far below peak plasma concentrations measured in patients after intravenous bolus injections However, the ratio between the two concentrations was significantly lower for atracurium and mivacurium as compared with succinylcholine ($P < 0.05$).

Discussion

The intradermal stimulation with muscle relaxants led to a dose-dependent increase of histamine and tryptase release from dermal skin, causing vasodilatation as well as itch or pain. Although there are reports about functional differences among mast cells isolated from different anatomic sites in response to muscle relaxants,[17,23] cutaneous mast cells are commonly used for the evaluation of mast cell degranulating potencies of different drugs.[10,18,19]

Fig. 3. Itch and pain ratings during intradermal stimulation with each muscle relaxant. Data are expressed as mean ± SD.
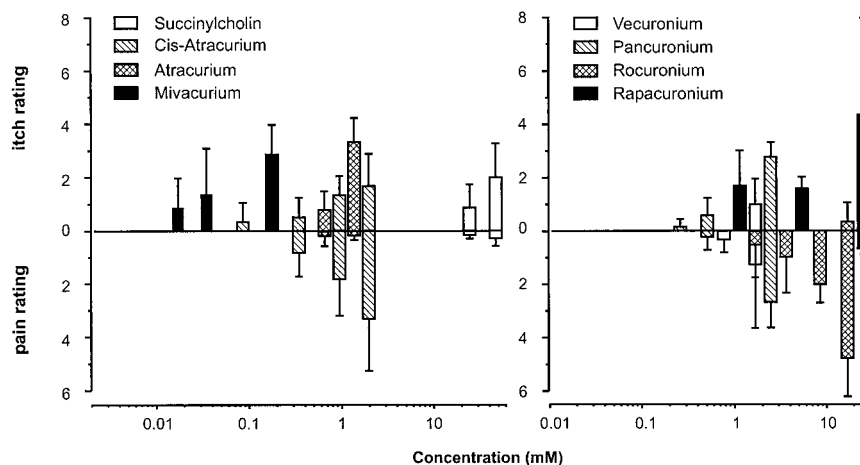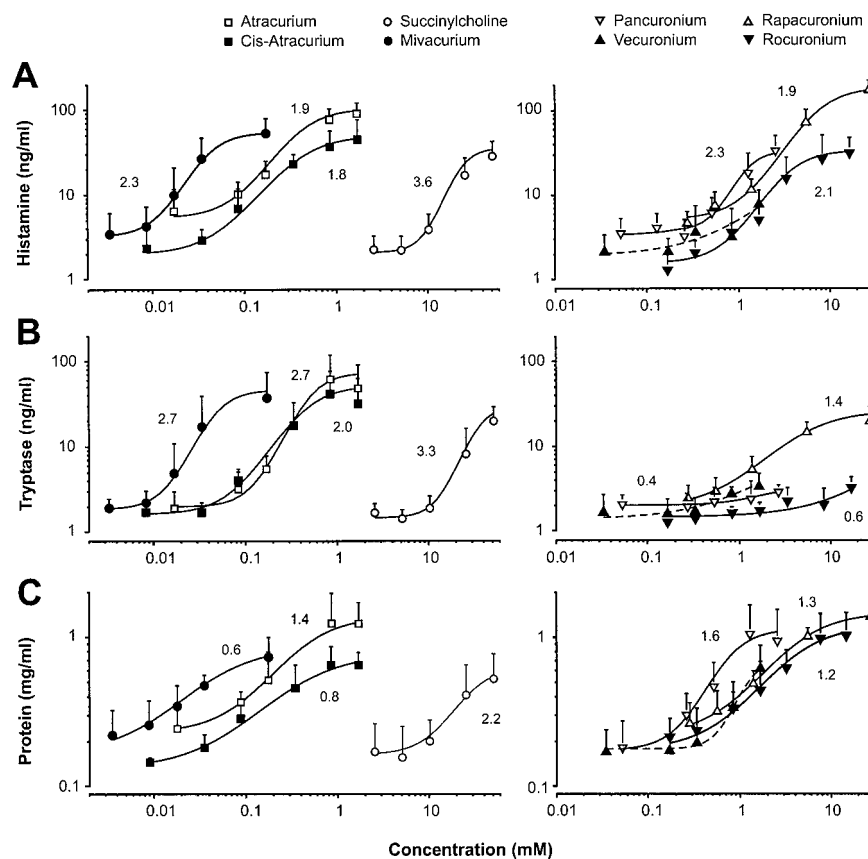
Fig. 4. Dose–response curves for histamine (*A*) and mast cell tryptase release (*B*) as well as protein extravasation (*C*) after intradermal stimulation with each muscle relaxant. Numbers indicate the Hill coefficient. A Hill coefficient greater than 1 indicates cooperativity, and higher values are combined with steeper shape of the sigmoidal dose–response curve (see Methods). Data are expressed as mean ± SD.

Succinylcholine- and Isoquinoline-type Muscle Relaxants

Succinylcholine and cisatracurium had the lowest potency of mast cell activation, confirming previous results.[17,24] However, despite their low potency, there might be an interaction with opioids or antibiotics, which can enhance the mast cell degranulation.[25]

Atracurium and even more mivacurium provoked mast cell activation very potently, again confirming previous *in vivo* studies in which systemic histamine release was measured.[10,26–28] Furthermore, the ratio between peak plasma concentration and ED_{50} for histamine release of mivacurium and atracurium was found to be significantly lower when compared with all other muscle relaxants (table 3). These observations are in accordance with previous *in vitro* studies[19] and are closely related to the clinically observed histamine release as well as local erythema and wheal formation after bolus injection of the respective muscle relaxant. It should be noted that the ratio between peak plasma concentration and ED_{50} values obtained with dermal microdialysis cannot be taken directly as a "safety factor" for histamine release because there is no information available about actual tissue concentrations after a bolus injection of muscle relaxants. In addition, we calculated the ED_{50} using the drug concentrations of the perfusate. Taking into account that the relative delivery (*i.e.*, the percentage of drug diffusing from the membrane into the tissue) is

roughly 30%, it can be assumed that also the tissue concentration close to the membrane reaches approximately one third of the perfusate concentration. Despite these limitations, ratios of peak plasma concentrations and ED_{50} values for histamine release reflect the probability of clinically observed histamine release for the various muscle relaxants.

In terms of molar concentration, atracurium has similar ED_{50} values for mast cell activation when compared with cisatracurium. Therefore, the lower incidence of anaphylactoid reactions induced by cisatracurium mainly depends on its higher potency as a muscle relaxant and thus lower clinically applied doses, rather than on isomer-specific effects. However, considering the higher

Table 2. Histamine/Tryptase Ratio

Muscle Relaxant	Stimulation	Wash-out
Succinylcholine	1.42 ± 1.09	1.32 ± 0.69
Cisatracurium	1.24 ± 0.55	1.21 ± 1.05
Atracurium	1.56 ± 0.66	1.32 ± 1.10
Mivacurium	1.39 ± 0.91	1.74 ± 1.55
Pancuronium	7.08 ± 2.13	0.65 ± 0.57
Rocuronium	7.44 ± 5.45	0.74 ± 0.72
Rapacuronium	6.37 ± 2.75	0.34 ± 0.16

Data are mean ± SD.

Stimulation = ratio of efficacies of histamine and tryptase release during stimulation; wash-out = ratio of efficacies of histamine and tryptase release during wash-out.

Fig. 5. Dose–response curves for local (*A*) and axon-reflex vasodilatation (*B*) and flare area (*C*) after intradermal stimulation with each muscle relaxant. Numbers indicate the Hill coefficient (see fig. 4) for each dose–response curve, respectively. Data are expressed as mean ± SD.
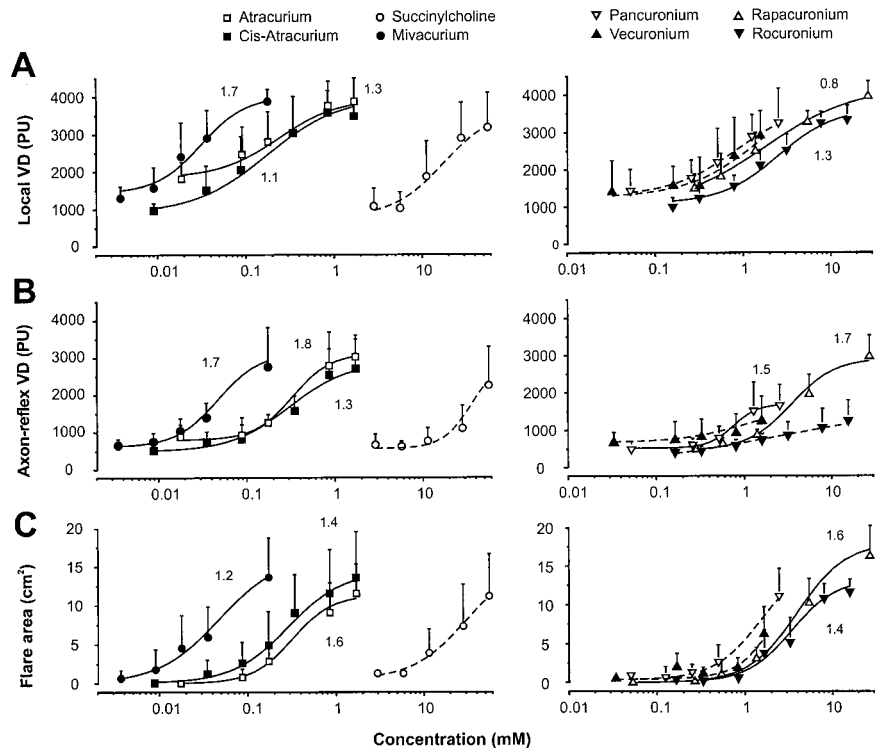
efficacy of atracurium for histamine release and protein extravasation, an isomer-specific effect on mast cell activation cannot be excluded completely.

For succinylcholine and the isoquinolines, dose–response curves for the vascular and sensory effects entirely fitted the histamine and tryptase release. This result suggests that mast cell activation is the main mechanism for muscle relaxant–induced vasodilation, protein extravasation, and neurogenic inflammation. Given the parallel results of the different vascular parameters and mediator release, it might appear redundant to assess all of them simultaneously for this group of relaxants.

Steroid-type Muscle Relaxants

In contrast to isoquinoline-type relaxants, the aminosteroids differed markedly in their pattern of sensory and

vasoactive effects and also their ability to activate mast cells. Rocuronium induced marked pain sensation, whereas mast cell activation was less pronounced. This result confirms clinical observations of withdrawal reactions induced by injection of rocuronium, which presumably reflect pain-induced reflexes.[29,30] More prominently, all of the tested aminosteroids (vecuronium, pancuronium, rocuronium, and rapacuronium) evoked a rapid histamine release that was not accompanied by a simultaneous increase in mast cell tryptase. Interestingly, the ratio of histamine to mast cell tryptase, which was between 1.24 and 1.56 for succinylcholine and the isoquinolines, varied only in a narrow range between 6.37 and 7.44 for the different aminosteroids. This result is surprising, because it is generally held that mast cell activation leads to a simultaneous release of the two stored mediators.[9] Other sources for the intracutaneous

Table 3. Comparison with *In Vivo* Data

Muscle Relaxant	Intravenous Bolus (mg/kg)	Plasma Concentration (µM)	ED$_{50}$ Histamine (µM)	Plasma Concentration/ ED$_{50}$ Histamine
Succinylcholine	1	83.0	23,642	285
Cisatracurium*	0.1	0.8	359	446
Atracurium	0.5	4.0	373	93†
Mivacurium	0.15	6.4	41	6†
Pancuronium‡	0.1	2.7	881	323
Rocuronium	0.6	11.5	2,998	261
Rapacuronium	1.5	29.5	7,939	269
Vecuronium	0.28	7.8	> 1,568	> 200

Peak plasma concentrations observed after bolus injection of the muscle relaxants in patients are compared with the concentrations required to provoke half-maximum histamine release (ED$_{50}$ histamine). Peak plasma concentrations were derived from references 43–48.

* Extrapolated from atracurium concentration.   † Ratios for atracurium and mivacurium were significantly lower, as compared with succinylcholine.   ‡ Extrapolated from reference 44 (applied dose was 0.05 mg/kg).

histamine comprise basophiles[31] and keratinocytes.[32] However, the absolute amount of basophils in the dermis is too low to explain the high histamine concentrations observed in our study. Another explanation could be a differential release of tryptase and histamine from mast cells. Histamine release without release of mast cell tryptase has been observed in mastocytosis patients.[33,34] The lack of mast cell degranulation after intradermal injection of vecuronium in humans has already been confirmed by electron microscopy.[18] Vecuronium injection left the mast cells intact, whereas after control injection of atracurium, the mast cells were degranulated. The macroscopic wheal reaction did not differ between the two injections. These results fit nicely to our observation that aminosteroids provoke a rapid release of histamine without concomitant tryptase degranulation leading to wheal and flare reactions, but not to a simultaneous increase in local tryptase concentration.

Surprisingly, intradermal stimulation with the aminosteroids led to a delayed and long-lasting increase in tryptase concentration. Rapacuronium induced this delayed increase most potently. This result may well be of major clinical importance. Rapacuronium can induce bronchospasm without histamine increase, and no correlation was found between mean arterial pressure decrease and systemic histamine concentrations.[35] The incidence of pulmonary side effects was reported to be 13.5% or even 18.5% after rapid sequence induction by rapacuronium with 2 mg/kg or 2.5 mg/kg, respectively.[36] These observations are at variance with the low histamine-releasing activity determined in our study. Therefore, other mediators responsible for these adverse reactions have to be taken into account. More than just a marker of mast cell activation, tryptase has been shown to specifically activate a subpopulation of proteinase activated receptors (PAR-2). PAR-2 activation leads to bronchoconstriction of intrapulmonary bronchi in the guinea pig[12] and has been hypothesized to be a major mediator for inflammation and bronchoconstriction in asthma.[37,38] In addition, PAR-2 receptors have been found in human vascular smooth muscle cells[39] and vascular endothelial cells.[40] Their activation led to marked hypotension in rat.[41,42] Thus, bronchoconstriction and hypotension after relaxant administration might be attributed to activation of PAR-2 receptors by tryptase rather than histamine.

It should be noted that efficacy values for the histamine and tryptase release of pancuronium, rocuronium, and almost certainly vecuronium were significantly smaller than those observed for rapacuronium. Taking into account the similar structural formula, it cannot be excluded that this mismatch might be caused by different solvents rather than the aminosteroid itself.

In conclusion, time courses of histamine and tryptase release by muscle relaxants revealed significant differences in mast cell activation. For succinylcholine and the

isoquinolines, dose–response curves for the vascular and sensory effects entirely fitted the histamine and tryptase release. In contrast, aminosteroids evoked a rapid histamine release that was accompanied by a delayed increase in tryptase. More than just being a marker for delayed degranulation of mast cells, tryptase, by activating PAR-2 receptors, might well be a pathogenetic factor for relaxant-induced histamine-independent bronchoconstriction and hypotension. It will therefore be of major interest to elucidate the mechanisms by which the different classes of muscle relaxants provoke mast cell activation and mediator release.

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