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THE INTRANASAL ABSORPTION OF THE QUATERNARY AMMONIUM COMPOUNDS NEOSTIGMINE BROMIDE AND TUBOCURARINE CHLORIDE IN THE RAT

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ABSTRACT

The systemic absorptions of the quaternary ammonium compounds, neostigmine bromide and (+)-tubocurarine chloride, from the nasal cavity were compared in the adult male rat with those obtained by the oral and intravenous routes. By the oral route, neostigmine bromide was absorbed to a limited extent and tubocurarine chloride demonstrated undetectable plasma levels. In contrast, use of the intranasal route resulted in plasma drug levels that were either significantly higher or readily detectable. Greater systemic absorptions and, thereby, improved bioavailabilities were achieved by coadministering the title drugs with sodium glycocholate, a surfactant, or with amastatin, an aminopeptidase inhibitor. For both drugs, bioavailabilities in the presence of amastatin were greater than in the presence of sodium glycocholate ($\geq$90% vs. $\geq$70%). Differences in the rate and extent of absorption of the quaternary ammonium compounds across the nasal mucosa appeared to reflect differences in molecular weight.

INTRODUCTION

Due to their highly ionized state at physiological pH and limited lipid solubility, certain therapeutically important quaternary ammonium compounds do not easily cross lipid membrane barriers (1). Not surprisingly, these compounds are often very poorly and irregularly absorbed from the gastrointestinal tract when given orally, and are devoid

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of significant effects on the central nervous system when administered parenterally (1-3). For such molecules to attain pharmacologically effective plasma levels they need to be delivered by the parenteral route, or in very large doses if given orally (3).

The nasal mucosa represents an easily accessible and potentially effective route for the systemic administration of drugs. Since it is anatomically composed of a ciliated epithelium coated with numerous microvilli and is underlined by a rich and extensive vascular and lymphatic network (4,5), it will offer drugs a large absorptive surface area and a direct entry to the systemic circulation (6). Numerous studies in humans and laboratory animals have shown transnasal delivery to be a desirable and reliable means of systemically administering drugs whose oral administration is unsatisfactory, impractical or compromised, or which may lead to considerable variation in plasma drug levels (7). Furthermore, on several instances use of the nasal cavity has provided a systemic absorption greater than that obtained through oral delivery and equivalent to that derived by the intravenous route (8). Even in those instances when drug absorption through the nasal mucosa is suboptimal, this route may still be a possibility when coupled with an absorption promoting agent (9-11).

Using an *in vivo* rat model for intranasal delivery and neostigmine bromide and (+)-tubocurarine chloride as model drugs, this study was aimed at evaluating the nasal absorption of quaternary ammonium compounds of differing molecular weights and oral absorption characteristics, with and without the addition of selected adjuvants, and at comparing the ensuing plasma pharmacokinetic data with those derived after oral and intravenous deliveries. At present, information on the nasal absorption of quaternary ammonium compounds appears to be quite limited.

EXPERIMENTAL

Animals and Materials

All experiments were carried out with male Sprague-Dawley rats (Taconic Farms, Germantown, NY), 250-300 g in weight, acclimated for 7 days, and fasted for at least 14 hr before the experiments. Experimental groups consisted of 5 rats each. All drugs and absorption enhancers used in the study (neostigmine bromide, (+)-tubocurarine chloride, urethane, sodium glycocholate, amastatin hydrochloride, and sodium heparin) were obtained from Sigma Chemical Co., St. Louis, MO. Additional reagents and chemicals were of analytical reagent or HPLC grade, and were used as received.

Treatments

Treatment solutions were prepared freshly in physiological saline, in 1% sodium glycocholate in physiological saline, or in 0.015% amastatin hydrochloride in physiological saline. Drug doses were 0.1 mg/kg for neostigmine bromide and 1 mg/kg for (+)-tubocurarine chloride. Intravenous injections were made through the tail vein at a volume of 300 μL/kg. Oral treatments were performed using an oral feeding needle and a volume of 1 mL/kg. Intranasal absorption studies were conducted as described earlier (12), on rats previously anesthetized with an intraperitoneal dose of urethane (1 g/5 mL/ kg) 15 min prior to a drug treatment. Treatment solutions were delivered into the nasal cavity, in a 300 μL/kg volume, via a piece of PE-20 polyethylene tubing affixed to a 100 μL microsyringe. The animals were kept lying on their backs and under a heat lamp throughout the experiments.

Blood Samples and Drug Assays

Blood samples were collected periodically into heparinized test tubes from a polyethylene cannula inserted into the femoral artery. Plasma samples were separated by immediate centrifugation, transferred to stoppered silanized tubes, and stored at 20°C until their analysis for drug content. Plasma (+)-tubocurarine levels were determined by the HPLC method of Meulemans et al. (13) with appropriate adjustments. Samples were extracted using a modification of the solid phase method of Avram et al. (14) using Supelclean LC-18 extraction tubes (Supelco, Inc., Bellefonte, PA) and elution with the HPLC mobile phase. Plasma neostigmine levels were measured by a modification of the HPLC method of De Ruyter et al. (15).

Analysis of Data

Plasma pharmacokinetic parameters were calculated based on a one-compartment model. A commercial statistical software package (PROC NOLIN program of PC SAS/STAT, SAS Institute Inc., Cary, NC) was used for this purpose. Results are expressed as the mean ± SEM of 5 determinations. Intergroup differences were established by Student's t-test, one way analysis of variance (ANOVA) and Dunnett's test. Differences were considered statistically significant at $p<0.05$.

RESULTS

Intranasal studies were conducted using a previously validated *in vivo* rat model that does not require surgery (12). To verify the results of an earlier study indicating that the rate and extent of absorption of several hydrophilic drugs from the nasal cavity is related to their molecular weight (16-18), the test drugs were selected to reflect differing molecular sizes, i.e., 223.3, neostigmine ion, and 610.7, tubocurarine ion. The doses used were based on those reported for humans (1-3). The dosing volumes were selected after preliminary comparisons of the area under the plasma level vs. time curve (AUC) and absolute bioavailability (F) data for graded dosing volumes in the range 50-300 μL/kg. Sodium glycocholate was used at a concentration previously found not to cause significant hemolysis, nasal protein leaching, ciliotoxicity, and/or histological evidence of mucosal damage (9,19-22). The concentration of amastatin was within the range used for the nasal delivery of insulin and other peptides of therapeutic interest (23).

The time course of the mean plasma levels of (+)-tubocurarine chloride and neostigmine bromide for the various treatments and routes of administration are shown in Figures 1 and 2, respectively. The pharmacokinetic data derived from these levels are summarized in Tables 1 and 2. Bioavailability results are presented in Figure 3.

After oral administration, (+)-tubocurarine chloride was not detected in the plasma throughout the experiment; hence, the oral bioavailability could not be evaluated. By the nasal route, the peak plasma level (C_{max}) occurred at 55 min postdosing and it was about one-fifth the value obtained intravenously. Relative to the parenteral route, intranasal delivery of (+)-tubocurarine chloride produced a markedly shorter (24%) plasma half-life ($t_{1/2}$), and AUC and F values that were about one-half lower. In the presence of glycocholate, the absorption rate (k_d), time to peak plasma level (t_{max}), and C_{max} were similar to those obtained from the formulation in saline solution alone; however, the $t_{1/2}$ was prolonged by 24% ($p<0.05$), and both the AUC and F were markedly enhanced (both by 42%). Substituting amastatin for sodium glycocholate brought about a faster k_d (219%), a nonsignificant (4%) lengthening of the $t_{1/2}$, higher C_{max} (162%), AUC (82%) and F (82%) values, and a much more rapid attainment of the C_{max} (<5 min).

Unlike (+)-tubocurarine chloride, neostigmine bromide was slowly absorbed from the gastrointestinal tract, with the C_{max} occurring in 39 min, and F = 15%. Compared to the oral route, intranasal dosing led to an 8-fold faster k_d, a faster t_{max} (20 min), a 4-fold

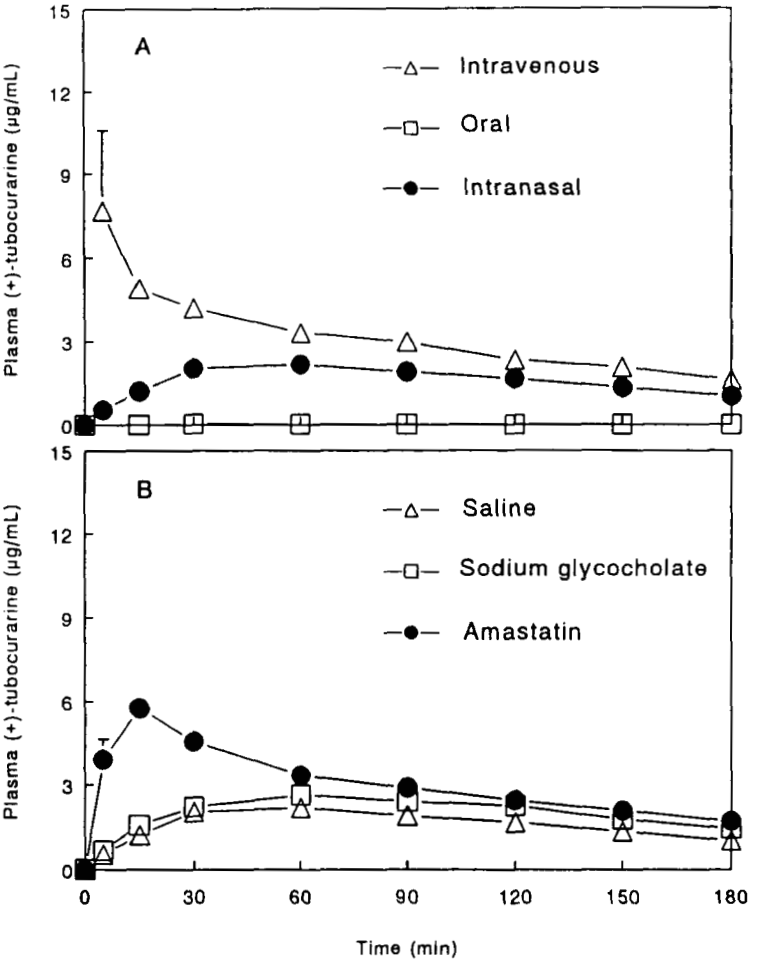

Figure 1. Temporal changes in plasma tubocurarine levels after the administration of a 1 mg/kg dose of tubocurarine chloride to rats (A) by intravenous, intranasal and oral routes, (B) by intranasal route with and without an adjuvant. Each point represents the mean for 5 rats. Vertical lines represent SEM.

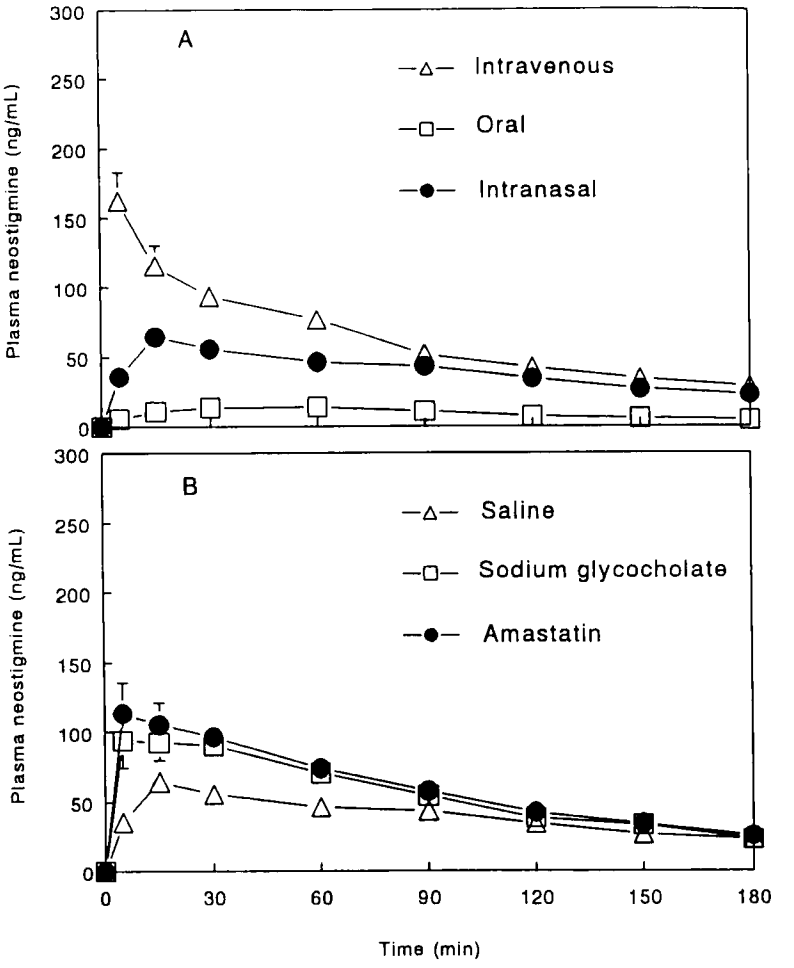

Figure 2. Temporal changes in plasma neostigmine levels after the administration of a 0.1 mg/kg dose of neostigmine bromide to rats (A) by intravenous, intranasal and oral routes, (B) by intranasal route with and without an adjuvant. Each point represents the mean for 5 rats. Vertical lines represent SEM.

Table 1

Plasma pharmacokinetic data for (+)-tubocurarine (1.0 mg/kg) after intravenous, oral and intranasal (with and without an absorption enhancer) deliveries[a-c]

Route	K_a (min^{-1})	K_e (min^{-1})	$t_{1/2}$ (*hr*)	t_{max} (*min*)	C_{max} (μg/mL *min*)	AUC (μg/mL *min*)
Intra-venous	--	0.0061 ±0.0003	1.89 ±0.25	--	--	854.75 ±52.49
Oral	--	--	--	--	--	--
Nasal	0.0352 ±0.0013	0.0080 ±0.0006	1.44 ±0.11	54.48 ±8.64	2.20[d] ±0.43	425.00 ±21.92
Nasal + SG	0.0351 ±0.0048	0.0064[d] ±0.0006	1.78[d] ±0.17	59.24[d] ±10.18	2.66 ±0.35	605.14[d] ±41.39
Nasal + AMA	1.1227[e] ±0.1083	0.0077[e] ±0.0007	1.50[e,f] ±0.93	4.47[e] ±0.73	5.77[e] ±0.42	775.06[e,f] ±35.52

[a]SG = sodium glycocholate (1%), AMA = amastatin (0.015%).

[b]Intravenous V_d = 0.2 ± 0.01 L/kg; C_o = 4.98 ± 1.02 μg/mL.

[c]Comparisons were made using ANOVA and Duncan's multiple range test.

[d]Comparisons vs. nasal ($p<0.05$).

[e]Comparisons vs. nasal + SG ($p<0.05$).

[f]Comparisons vs. intravenous ($p<0.05$).

increase in C_{max}, a longer $t_{1/2}$ (1.83 hr), and a 5-fold increase in F. Adding glycocholate to the nasal formulation led to a still faster k_d, a faster t_{max} (9 min), a shorter $t_{1/2}$ (1.35 hr), a more than 6-fold increase in C_{max}, and AUC and F values that were 20% higher than from a formulation in saline alone. Using amastatin in place of glycocholate magnified the effects achieved with the latter adjuvant. In this case, the k_d was more than 4-fold faster, the $t_{1/2}$ was shorter, the t_{max} was faster (6 min) and the C_{max} was almost doubled. Relative to the formulation in plain saline, amastatin increased F by 28%.

DISCUSSION

The bulk of work on the intranasal delivery of hydrophilic molecules has dealt with peptides, proteins and vitamin B_{12}, all of which exhibit little tendency to partition into

Table 2

Plasma pharmacokinetic data for neostigmine (0.1 mg/kg) after intravenous, oral and intranasal (with and without an absorption enhancer) deliveries[a-c]

Route	K_a (min^{-1})	K_e (min^{-1})	$t_{1/2}$ (hr)	t_{max} (min)	C_{max} (μg/mL)	AUC (μg/mL·min)
Intra-venous	-- --	0.0087 ±0.0007	1.33 ±0.11	--	--	14.87 ±1.32
Oral	0.0524 ±0.0069	0.0101 ±0.0011	1.14 ±0.12	38.93 ±3.02	0.015 ±0.003	2.16 ±0.13
Nasal	0.1667[d] ±0.0256	0.0063[d] ±0.0008	1.83[d] ±0.23	20.39[d] ±2.51	0.061[d] ±0.011	10.95[d] ±0.13
Nasal + SG	0.4395[e] ±0.0721	0.0082[e] ±0.0005	1.42[e] ±0.09	9.24[e] ±0.98	0.100[e] ±0.025	13.26[e] ±1.52
Nasal + AMA	0.7126[f] ±0.1154	0.0085[f] ±0.0002	1.35 ±0.03	6.28[f] ±0.42	0.114 ±0.032	14.12 ±1.46

[a]SG = sodium glycocholate (1.0%), AMA = amastatin (0.015%).

[b]Intravenous V_d = 0.81± 0.07 L/kg; C_o = 0.124 ± 0.021 μg/mL.

[c]Comparisons were made using ANOVA and Duncan's multiple range test.

[d]Comparisons vs. oral ($p<0.05$).

[e]Comparisons vs. nasal ($p<0.05$).

[f]Comparisons vs. nasal + SG ($p<0.05$).

the nasal mucosa (4,6). By comparison, very few studies have been addressed to the intranasal delivery of highly water-soluble, highly ionized drugs with low oral bioavailability (16,17,24); this paucity of information extends to quaternary ammonium compounds, for which the only published report appears to be one on the antiarrhythmic agent clofilium tosylate in the rat (24).

The two quaternary ammonium compounds examined here showed some significant differences in absorption following their oral and intranasal administrations (Figures 1-3, Tables 1and 2). By the oral route, only neostigmine was detected in the plasma between 0-180 min postdelivery. By the intranasal route, neostigmine was absorbed more rapidly, and it reached its C_{max} in about one-half the time needed by (+)-tubocurarine.

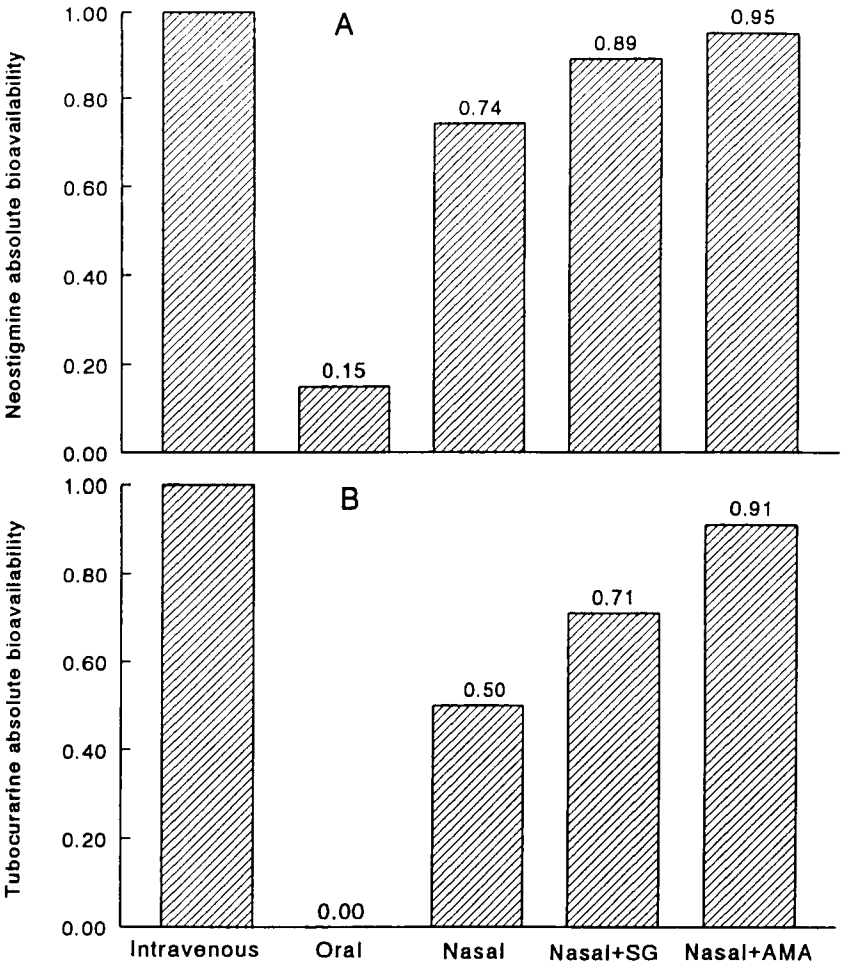

Figure 3. Comparison of absolute bioavailabilities after oral and intranasal (with and without an adjuvant) administrations of (A) neostigmine bromide, 0.1 mg/kg, and (B) tubocurarine chloride, 1 mg/kg to rats. Key: SG = sodium glycocholate; AMA = amastatin. Values represent the mean for 5 rats.

Similarly, the absolute bioavailability of neostigmine was 1.5-fold greater than that of (+)-tubocurarine. A comparison of these bioavailabilities with that reported for a 1.2 mg/kg intranasal dose of clofilium tosylate (F = ca. 70%) (23) indicates that the rate and extent of the nasal absorption of quaternary ammonium compounds correlates well with their molecular sizes, but in an inverse manner. Thus, the decreasing order of bioavailabilities neostigmine ion > clofilium ion > tubocurarine ion is in agreement with their increasing molecular weights, i.e., 223.3, 338.5, and 610.7, respectively. Such a correlalation has been previously demonstrated with model hydrophilic substances possessing molecular weights in the range 190-70,000 daltons, with a sharp fall off in nasal mucosal uptake occurring at values above 1,000 (16,24). However, other possible determinants of variability in nasal ab-sorption among highly hydrophilic drugs may stem from differences in molecular rigidity (5), polarity (26), net charge at physiological pH (24), and hydrophobic balance (25). Accordingly, one would predict that the smaller, more soluble and flexible, monocationic neostigmine ion will exhibit a greater nasal absorption and, thus, a greater bioavailability than the larger, less soluble, more rigid, dicationic tubocurarine ion. The present findings agree with these predictions.

Earlier results with hydrophilic compounds such as sodium cromoglycate (MW 512) (16) and inulin (MW 5,200) (17), suggest that quaternary ammmonium compounds penetrate the nasal mucosa via a nonspecific diffusion process that may take place, at least partly, through cell-cell junctions and spaces between cells in the nasal membrane (9,18,28,29) and that these aqueous channels pose a size restriction on permeability (25). However, by analogy with the mucosa of the ileum and gallbladder, the nasal cavity is viewed as a truly selective barrier rather than as a simple diffusion pathway (27). Using this concept, many substances have been tested to alter the permeability of the nasal mucosa as a means of enhancing solute absorption (4,9-11), with bile salts and their conjugates representing the most popular absorption enhancers (10). In the present study, the concurrent intranasal administration of neostigmine or (+)-tubocurarine salts with sodium glycocholate resulted in a marked increase in the bioavailability of both drugs (>88%, neostigmine; >70%, (+)-tubocurarine) (Figure 3); but changes in k_d and t_{max} relative to the nasal delivery from a formulation in plain saline were only seen with neostigmine. It has been proposed that bile salts act as penetration enhancers by inhibiting the nasal activity of leucine aminopeptidase while exerting little effect on membrane integrity (21). Therefore, it was hypothesized that these surfactants might influ-

ence aqueous junctions to decrease cell-cell adhesion (18), to cause the channel to enlarge (25), or to become more hydrophilic (25). To confirm this possibility, the quaternary ammonium compounds were administered together with amastatin, an aminopeptidase inhibitor used to promote the nasal absorption of peptides (23,30). As shown in Figure 3, the addition of amastatin affected both the fraction of drug absorbed (as measured by the AUC) and the systemic bioavailability, to an extent that exceeded those obtained in the presence of sodium glycocholate. Since amastatin possesses a much greater inhibitory activity on membrane protein enzymes than does the glycocholate, it would appear that nasal proteins represent a barrier to the absorption of drugs from the nasal cavity; but inhibition of proteolytic activity as the reason for the absorption enhancement is ruled out because of the nonpeptide nature of the test drugs. Rather, it is possible that amastatin favors nasal drug absorption by influencing the conformation of proteins apposed to constitute aqueous channels so as to create larger pores (31), or by disturbing the dynamic equilibrium among proteinases, peroxidases, and proteinase inhibitors of the nasal mucosa (30).

In conclusion, the intranasal delivery of two quaternary ammonium compounds such as neostigmine bromide and (+)-tubocurarine chloride yielded systemic absorptions and bioavailabilities that were far greater than those attained by the oral route. Furthermore, the bioavailability of these compounds was improved upon their coadministration with the absorption enhancers sodium glycocholate, a surfactant, and amastatin, a peptidase inhibitor. The extent of the intranasal absorption was found to be inversely related to the molecular weight of the quaternary ammonium compound.

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