Effects of *N*-Demethylated Carbachol on Iris and Ciliary Muscles

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Abstract □ The tertiary nitrogen derivative of carbachol, *N*-demethylated carbachol, was synthesized to produce a drug that could be used as an alternative to pilocarpine in the treatment of open-angle glaucoma. Unlike pilocarpine, *N*-demethylated carbachol appeared to be stable in alkaline solutions. Since pilocarpine has to be dissolved in an acidic medium (pH 5.5) to prevent its destruction, <10% of its molecules are in an unionized form. On the other hand, *N*-demethylated carbachol is very stable at pH values higher than 7.4 and thus has more molecules (>10%) in an unionized, penetrable form than does pilocarpine in high pH media. *N*-Demethylated carbachol acted as a full agonist on isolated dog iris and ciliary muscle preparations, whereas pilocarpine produced only 49 and 38% of the maximum tissue responses, respectively. These findings suggest that *N*-demethylated carbachol may be able to affect the outflow facility to a greater extent than is achievable with pilocarpine at high doses.

Keyphrases □ Carbachol, *N*-demethylated derivative—effect on iris and ciliary muscles, treatment of open-angle glaucoma □ *N*-Demethylated carbachol—effect on iris and ciliary muscles, treatment of open-angle glaucoma □ Antiglaucoma agents—*N*-demethylated carbachol, effect on iris and ciliary muscles, treatment of open-angle glaucoma □ Ophthalmic preparations—*N*-demethylated carbachol, effect on iris and ciliary muscles, treatment of open-angle glaucoma

Pilocarpine is one of the oldest antiglaucoma agents used in ophthalmology (1). It exerts its action primarily through the increase of aqueous humor outflow (2). However, it induces some side effects such as miosis, exocrine gland secretions, and local irritations, which prevent it from being used continuously in some patients (3).

N-Demethylated carbachol recently was reported to possess a potency similar to that of pilocarpine in its ability to lower the intraocular pressure of glaucomatous dogs but to cause only minor miotic effects and to be devoid of other untoward actions produced by pilocarpine (4). Therefore, the effects of these two antiglaucoma drugs in the isolated ocular tissues, iris, and ciliary muscles were compared. The pKa value of *N*-demethylated carbachol was determined, and the ionization curves were constructed for comparison with its penetrability.

EXPERIMENTAL

Chemistry—*N*-Demethylated carbachol hydrochloride was synthesized in this laboratory by a modification (4) of the method of Hazard *et al.* (5). Dimethylaminoethanol was refluxed with an excess of ethyl carbamate in toluene for 2 hr to remove water by using a trap receiver. Aluminum methoxide was added to catalyze the reaction, and the reaction mixture was refluxed for an additional 12 hr. The reaction by-product, ethanol, was removed by distillation along with toluene at a rate of 2–5 ml/min for 10 hr. Fresh toluene was added periodically to replenish the lost volume. Aluminum methoxide then was filtered off while the reaction mixture was still hot.

The product, *N*-demethylated carbachol base, was distilled with fractional distillation under vacuum to avoid overheating. (The distillation temperature should not exceed 130° to avoid polymerization and reduction in the yield.) The base product then was converted into the hydrochloride salt with dry hydrogen chloride gas in ether. The final product was recrystallized with absolute ethanol. *N*-Demethylated carbachol was tritiated commercially[1] with a specific activity of 31 mCi/mmole. The ^{3}H-*N*-demethylated carbachol was stored as a dried powder and was dissolved in an aqueous medium immediately before use to avoid tritium exchange. Pilocarpine nitrate and carbachol chloride were purchased from commercial sources.

The stability of aqueous solutions of *N*-demethylated carbachol was studied with NMR spectroscopy. Free *N*-demethylated carbachol base and its salt were dissolved in heavy water for NMR analysis.

The acid dissociation constants, pKa, of *N*-demethylated carbachol and pilocarpine were determined with a potentiometric titration method. The standardized carbonate-free potassium hydroxide solution (0.1 *M*) was obtained commercially[2] for titration. The drug quantities employed were selected to achieve concentrations of 1×10^{-5} *M* at the point of half-neutralization. Values of pKa and their scatter were calculated as described in the literature (6).

Penetrability—^{3}H-*N*-Demethylated carbachol solution (25 μl of 0.128 *M* and 31 mCi/mmole at pH 7.4) was instilled into the eyes of conscious rabbits, and the eyelids were held closed for 1 min. Rabbits were killed at 10, 20, 60, and 180 min after instillation. The eyes were rinsed with saline to remove surface radioactivity, and the tissues were obtained in the order of aqueous humor, cornea, iris, and ciliary body. All tissues were weighed, digested with a solubilizer[3], and counted in a scintillation medium[4] with a scintillation counter[5]. The tissue uptake of ^{3}H-*N*-demethylated carbachol was expressed in picomoles per milligram of tissue. The amount of *N*-demethylated carbachol taken up by ocular tissues at the approximate peak time was tabulated.

Isolated Iris and Ciliary Muscle Preparation—Mongrel dogs, 13–17 kg, were sacrificed with an intravenous injection of pentobarbital sodium. The eyes were removed and placed on a moist gauze, and the iris was dissected according to a literature method (7). After removal of the iris, an incision was made in the sclera to expose the interior of the glove. The lens and adhering vitreous humor were removed, and a shallow incision was made in the uvea anterior to the ora serrata. A strip of ciliary muscle, 4–5 mm wide and approximately 25 mm long, was carefully lifted free of the sclera. Sections of iris and ciliary muscles were suspended in 6-ml organ baths containing a Ringer solution (119.8 m*M* NaCl, 4.02 m*M* KCl, 1.98 m*M* $CaCl_2$, 1.27 m*M* $MgSO_4$, 26.2 m*M* $NaHCO_3$, 1.0 m*M* KH_2PO_4, and 11.1 m*M* glucose at pH 7.4) oxygenated with 95% O_2–5% CO_2 at 32°.

A microdisplacement force transducer[6] was used to detect the muscle contraction, and the contraction was recorded on a polygraph[7]. The initial tension placed on the iris was 100 mg and that on the ciliary muscle was 300 mg. Effects of *N*-demethylated carbachol were compared with those of pilocarpine and carbachol. The maximum contraction induced by carbachol was designated as the 100% response, and other responses induced were calculated as a percent of that maximum response.

RESULTS

The NMR spectrum of *N*-demethylated carbachol dissolved in heavy water produced the expected chemical shifts and splitting patterns. The spectrum consisted of a singlet at δ 4.72 (NH_2), two triplets at δ 4.27 and 2.65 (CH_2CH_2), and another singlet at δ 2.27 [$N(CH_3)_2$] relative to Tiers' salt, $(CH_3)_3Si(CH_2)_3SO_3Na$. The NMR spectra of the salt and free base of *N*-demethylated carbachol obtained at regular intervals after the compounds were dissolved in heavy water remained unchanged at room temperature for at least 3 months.

[1] ICN Pharmaceuticals.
[2] Baker Chemical Co.
[3] NCS solubilizer, Amersham/Searle Corp.
[4] Biofluor scintillation medium, New England Nuclear Co.
[5] Model LS100, Beckman Instruments.
[6] Myograph A, Narco BioSystems.
[7] Physiograph, Narco BioSystems.

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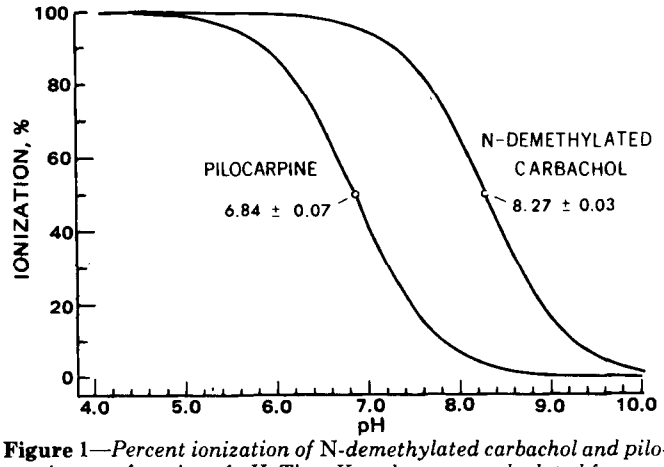

Figure 1—*Percent ionization of* N-*demethylated carbachol and pilocarpine as a function of pH. The pKa values were calculated from experimentally determined values at 25°* (n = 9).

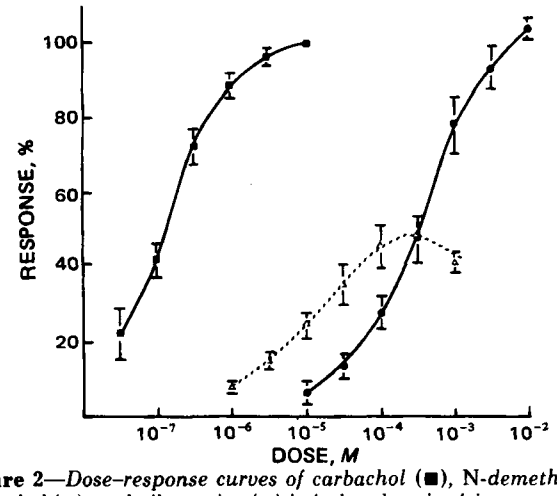

Figure 2—*Dose–response curves of carbachol* (■), N-*demethylated carbachol* (●), *and pilocarpine* (△) *in isolated canine iris preparations. Each point is the mean of four values, and the bars indicate standard errors.*

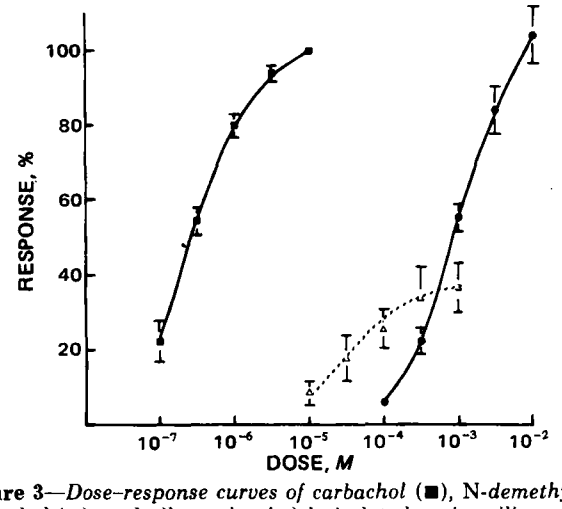

Figure 3—*Dose–response curves of carbachol* (■), N-*demethylated carbachol* (●), *and pilocarpine* (△) *in isolated canine ciliary muscle preparations. Each point is the mean of four values, and the bars indicate standard errors.*

The pKa values of *N*-demethylated carbachol and pilocarpine and the ionization curves constructed from these values are shown in Fig. 1. An additional pKa value for pilocarpine should be at 12.57 (8), but it was not observed in this laboratory. Titrations of *N*-demethylated carbachol revealed that this compound has a single pKa value of 8.27.

The penetrability of *N*-demethylated carbachol into the eyes is presented in Table I. The peak time for *N*-demethylated carbachol to reach intraocular tissues was ~20 min, and the penetrability was about the same as that of pilocarpine (9).

N-Demethylated carbachol contracted the isolated dog iris at doses of 1×10^{-5}–1×10^{-2} *M* (Fig. 2). The dose–response curve paralleled that of carbachol. Pilocarpine was more potent at lower doses (1×10^{-6}–1×10^{-4} *M*) than *N*-demethylated carbachol, but the maximum effect attainable with this drug was only 49% of that produced by carbachol or *N*-demethylated carbachol. Pilocarpine doses greater than 3×10^{-4} *M* decreased muscle tension.

The overall shape of the dose–response curves of *N*-demethylated carbachol, carbachol, and pilocarpine obtained with ciliary muscle was similar to that obtained with the iris preparation, except that the ciliary muscle appeared to be less sensitive to these agents (Fig. 3). The maximum response produced by pilocarpine was only 38% of that produced by *N*-demethylated carbachol or carbachol.

DISCUSSION

The usefulness of a drug as eye drops in the treatment of glaucoma depends primarily on its penetrability across the cornea. Although pilocarpine is a much less potent muscarinic agent than carbachol, it is more widely used than carbachol as an antiglaucoma agent because it can penetrate the cornea better than carbachol.

At the physiological pH of 7.4, 78% of pilocarpine is in the unionized, penetrable form (Fig. 1). However, pilocarpine is not stable at this pH because it undergoes base-catalyzed epimerization or is cleaved at the lactone ring (10, 11). For this reason, the pH of most ophthalmic preparations of pilocarpine is adjusted to 4.5–5.5 (12). At these pH values, >95% of the drug is ionized.

The ester linkage in *N*-demethylated carbachol is the same as that in carbachol, which is known to be quite stable in aqueous solution. This structure was confirmed in this laboratory with NMR studies. Thus, an ophthalmic solution of *N*-demethylated carbachol at pH 7.4 can be made.

Table I—Penetration of *N*-Demethylated Carbachol into Ocular Tissues

Tissue	Peak Time, min	Quantity at Peak, pmoles/mg	Tissue Weight, mg	Total Amount in Tissue, nmoles	Percent Absorbed[a]
Cornea	10	129[b]	64[c]	8.26	0.26
Iris	20	20	35	0.70	0.02
Ciliary muscle	20	15	27	0.41	0.01
Aqueous humor	20	21	287	6.03	0.20

[a] The calculation was based on the total amount of *N*-demethylated carbachol instilled (3.19 μmoles). [b] $n = 6$. [c] $n = 19$.

At pH 7.4, <90% of *N*-demethylated carbachol is ionized. Thus, in clinical situations (*N*-demethylated carbachol at pH 7.4, pilocarpine at pH 5.5 or less), *N*-demethylated carbachol could be more effective than pilocarpine because it has greater penetration. Moreover, a drug solution with a pH range of 6.6–8.7 may be instilled into the eye without producing unpleasant sensations (13). Therefore, it should be possible to increase the effectiveness of *N*-demethylated carbachol even further by administering it in solutions having pH values greater than 7.4 but less than 8.7. At pH 8.7, 70% of *N*-demethylated carbachol is unionized and penetrable.

In actual experimentation with ^{3}H-*N*-demethylated carbachol, the drug was absorbed to reach a concentration in the aqueous humor of 2.1×10^{-5} *M* 20 min after topical administration at pH 7.4.

Experiments with isolated iris and ciliary muscles demonstrated that *N*-demethylated carbachol has a greater intrinsic activity or efficacy than pilocarpine. However, pilocarpine possesses higher sensitivity to these muscles at lower doses (1×10^{-6}–1×10^{-4} *M* in the iris preparation and 1×10^{-5}–2×10^{-4} *M* in ciliary muscle). The combination of these two factors could explain the fact that, in *in vivo* studies, *N*-demethylated carbachol is as potent as pilocarpine in lowering the intraocular pressure but causes less miosis in the glaucomatous beagle eye.

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Effect of Low and High Relative Humidity on Metered-Dose Bronchodilator Solution and Powder Aerosols

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Abstract □ Physical properties of two metered-dose bronchodilator aerosols packaged as solutions and two aerosols packaged as finely ground powders were measured at low and high relative humidity. The aerodynamic size distribution and particle concentration were measured in real time using the single-particle aerodynamic relaxation time analyzer, which can measure the aerodynamic diameter of single suspended particles in the respirable size range. The count median aerodynamic diameter, the mass median aerodynamic diameter, the total particles per dose, and the total aerodynamic mass per dose were calculated. Significant increases were noted in the count median aerodynamic diameter for three aerosols and in the mass median aerodynamic diameter for two aerosols. The number of particles in the measured size range increased 3.6- and 4.1-fold for the droplet aerosols and 1.4-fold for the powder preparations. The aerodynamic mass per dose in the measured size range increased 5.7- and 11.4-fold for the droplet aerosols and 3.1- and 1.6-fold for the powder aerosols. These data indicate that all aerosols tested increased in size at high humidity and that aerosols dispensed as droplets may be more unstable than those dispensed as powders.

Keyphrases □ Bronchodilators—metered-dose solution and powder aerosols, effect of low and high humidity □ Aerosols—bronchodilators, metered-dose solution and powder preparations, effect of low and high humidity □ Asthma products—metered-dose solution and powder aerosols, effect of low and high humidity □ Metered-dose aerosols—bronchodilators, solution and powder preparations, effect of low and high humidity

Metered-dose bronchodilator aerosols are used widely to treat obstructive lung disease. The efficacy of these medications is determined by their quantity and site of pulmonary deposition. Factors that affect pulmonary retention and distribution are the inhalation technique, airway patency, and particle size.

BACKGROUND

The particle-size distributions of aerosols produced by some of these devices have been reported (1–3), but the effect of hygroscopicity on particle size was not included. Any particle may grow by water condensation or shrink by water evaporation, depending on the humidity and physical characteristics of the particle. Such changes may affect the mass and site of aerosol deposition in the respiratory tract. Although particle growth during exposure to high humidity such as that occurring in the respiratory tract has been predicted for therapeutic aerosols (4) and the effect of humidity on the size of propylene glycol aerosols has been described (5), there is no information documenting or quantitating the effect of high humidity on bronchodilator aerosols.

Measurement of aerosol particle size is most relevant for prediction of deposition when the size is expressed as the aerodynamic diameter, defined as the diameter of a unit density spherical particle having the same terminal settling velocity as the particle in question. Methods currently used to measure the aerodynamic size distribution of aerosols require particle impaction and subsequent analysis of the quantity deposited (4, 6). The instability of water-containing particles has been described (7), and size changes may occur in <1 sec (8). This instability precludes extrapolation of the particle-size measurement following precipitation of unstable particles to the particle size in the suspended state. Measurement in the suspended state is necessary to assess accurately the effect of humidity on the size of unstable particles.

The purpose of this study was to determine the effect of high humidity, similar to that encountered in the respiratory tract, on the particle-size distribution of four commonly used metered-dose aerosol devices. Humidity in the trachea and more distal airways generally is regarded to be in the 99.0–99.8% range (9). The mass median aerodynamic diameter and count median aerodynamic diameter (the sizes below which are 50% of the particles by mass and number, respectively), the geometric standard deviation (84.1% size divided by 50% size), the mass per dose, and the number of particles per dose were measured. A new device, the single-particle aerodynamic relaxation time analyzer (hereafter called the analyzer) that measures the aerodynamic diameter of single particles in real time, was used for these studies.

EXPERIMENTAL

The following medications were studied: isoproterenol hydrochloride[1] and isoetharine mesylate[2], both packaged as solutions, and metaproterenol sulfate[3] and isoproterenol sulfate[4], both packaged as finely ground powders. In each case, the commercially available preparation was used. Each medication was injected into an environmental chamber for subsequent sampling by the analyzer.

The analyzer (Fig. 1) and the theoretical basis for its operation were described previously (3, 10, 11). The sensing volume, in which the signal

[1] Isuprel Mistometer, Winthrop Laboratories, New York, N.Y.
[2] Bronkometer, Breon Laboratories, New York, N.Y.
[3] Metaprel, Dorsey Laboratories, Lincoln, Neb.
[4] Medihaler-Iso, Riker Laboratories, Northridge, Calif.

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