Antimuscarinic effects of stereoisomers of tropicamide on rabbit iris sphincter. P. N. Patil.

The antimuscarinic activity of optical isomers of tropicamide were compared on the isolated rabbit iris sphincter. The increasing concentrations of both the (−)- and (+)-isomer shifted the dose-response curve of carbachol to the right in a parallel fashion. The competitive reversible muscarinic blocking effects of both isomers were confirmed by pA2 plots. The pA2 values from the nonpigmented irides for (−)- and (+)-tropicamide were 7.88 and 6.18, respectively. Thus the (+)-isomer has only 1/3 the blocking activity of the (−)-isomer. Although both isomers are slightly less active in the pigmented iris, the blocking effect of the active (−)-tropicamide was readily reversed by washing, whereas reversal of this isomer’s effect from the pigmented iris was relatively slow.

Tropicamide (Mydriacyl) is a rapidly acting antimuscarinic drug widely used for producing cycloplegia and mydriasis of short duration. The chemical structure indicates an asymmetric center in the molecule. The possibility of two optical isomers is quite obvious. Clinically, the drug is used as a racemate which contains equal parts of the levo and the dextro isomers. The pharmacologic activity of racemic mixtures usually resides in one isomer. In other words, the pharmacologic effects of drugs are stereoselective. Availability of the optical isomers of tropicamide prompted us to investigate the muscarinic blocking effects of the stereoisomers in the iris.

Materials and methods. Both albino and nonalbino (black fur) rabbits weighing 2 to 3.5 kg were sacrificed by injecting sufficient air into the marginal ear vein. Eyes were rapidly removed, and the iris was dissected in an oxygenated physiologic salt solution having the following composition in millimole: NaCl, 118; KCl, 4.7; CaCl2, 2.5; MgCl2, 0.54; Na2HPO4, 1.0; NaHCO3, 25; and glucose, 11.0. A thread was passed through the iris and tied to a hook; the opposite end of the tissue was connected by means of a silk thread to a force-displacement transducer. The isolated iris was suspended in a 10 ml organ bath containing the physiologic salt solution at 37°C. Throughout the experiment a baseline tension of 150 to 200 mg was maintained, and the change in tension developed by the iris sphincter in response to drugs was recorded on a Grass polygraph. During a 50 min equilibration period the tissue was washed at regular intervals; following this, two cumulative dose-response curves of carbachol were obtained. The first dose-response curve was done in the absence of antagonist, following which the tissue was thoroughly washed. The tissue was then exposed for 60 min to the antagonist, and a second dose-response curve to carbachol was obtained in the continued presence of the antagonist. pA2 values (which can be defined as a negative log molar concentration of an antagonist which shifts the dose-response curve of an agonist by twofold) were calculated according to the method of Arunlakshana and Schild. Dose-ratio (which is defined as a ratio of ED50 of carbachol in the presence and absence of antagonist) was used to calculate pA2 plots. The pA2 value indicates an apparent affinity of the antagonists to the receptor. A potent antagonist produces a high pA2 value.

In another series of experiments on the iris, re-
Fig. 1. Relative muscarinic blocking effects of (-)- and (+)-tropicamide at a single concentration. Dose-ratio is a ratio of the ED50 of carbachol with and without the blocker.

Fig. 2. Plot to obtain pA2 values of the isomers of tropicamide from the nonpigmented and pigmented iris. Higher pA2 values indicate greater affinity of the antagonists. On the basis of pA2 values from nonpigmented iris, the (+)-isomer has only 1/60 the blocking activity of the (-)-tropicamide. Note that at all concentrations the isomers are slightly less active in the pigmented iris.

covery from the muscarinic blockade was studied by testing the sensitivity of the tissue to a dose of carbachol at regular intervals. A control response to carbachol ED50 (10^-3M) was obtained, and the tissue was thoroughly washed and incubated with a dose of a blocker for 60 min. Sensitivity of the tissue to the dose of carbachol in the presence of a blocker was ascertained. The tissue was washed three times within 5 min, and 10 min after the last wash, the tissue sensitivity to carbachol was tested. The procedure was repeated several times.

Since the tropicamide is resistant to hydrolysis by atropine sterases from rabbit, atropine esterase-positive animals were used.

Levo tropicamide HCl, sp. rotation -95.05 degrees, and dextro tropicamide HCl, sp. rotation +97.78 degrees, were obtained from Alcon Laboratories, Fort Worth, Texas, through the courtesy of Dr. L. DeSantis. Since the isomers are highly hygroscopic, precautions were taken to avoid moisture. In the initial experiments drug solutions were freshly prepared every day. How-
ever, specific optical rotations of the 2-week-old and the freshly prepared solutions were identical. This indicates that the isomers do not undergo racemization. Dilutions of carbachol chloride (Aldrich) in saline were carried out from the stock solutions. Throughout the manuscript, \textit{levo} and \textit{dextro} isomers will be designated by signs (−) and (+), respectively.

\textbf{Results.} Although (+)-tropicamide when compared with the (−)-isomer is a much weaker muscarinic blocker, both isomers in a concentration-dependent fashion shifted the dose-response curve of carbachol to the right. The dose-ratio at $10^{-6}$M (−)-tropicamide was 1.5, and that at $10^{-4}$M (−)-tropicamide was 52. The blocking activity of (+)-tropicamide was tested at $3 \times 10^{-7}$M, $10^{-6}$M, and $10^{-5}$M concentrations. At the highest concentration tested the (+)-isomer gave a dose ratio of 9, whereas at comparable concentration the dose-ratio with the (−)-isomer was 300 (Fig. 1).

At three different concentrations both isomers were also tested on the pigmented iris from the nonalbino animals. As compared to the effect seen in the nonpigmented iris, at all concentrations the (−) or (+)-tropicamide was less effective in the pigmented iris. For example, (−)-tropicamide at $3 \times 10^{-7}$M produced a dose-ratio of 22 from the nonpigmented iris, whereas the ratio at the same concentration of the isomer from the pigmented iris was only 10. Thus the (−)-tropicamide has apparently twice the blocking activity in the nonpigmented iris as compared to the pigmented iris.

\textit{pA}_2 plot. Data are illustrated in Fig. 2. The plotting method provides a standard and accurate procedure for comparing the potencies of antagonists. According to Arunlakshana and Schild, the slope of one of the \textit{pA}_2 plots indicates a competitive antagonism. The slope values of the plots found experimentally are fairly close to 1. In the nonpigmented iris, based on \textit{pA}_2 values, the (+)-isomer has only $\frac{1}{10}$ the blocking activity of the (−)-isomer. On the basis of similar comparisons, (+)-tropicamide has $\frac{1}{12}$ the blocking activity of the (−)-tropicamide in the pigmented iris.

\textit{Recovery from the blockade}. The concentration of (−)-tropicamide ($3 \times 10^{-7}$M), which produces near complete blockade of $10^{-4}$M carbachol, could be easily washed off (t½ <15 min) from the nonpigmented iris, whereas the recovery of this isomer under the same conditions from the pigmented iris is slow (Fig. 3). The t½ recovery for the response is approximately 30 min.

The data obtained with the tropicamide isomers are in line with the well-known stereoselectivity exhibited by muscarinic receptors. Previously, when the isomers of muscarinic agonists or antagonists were compared, the stimulant or blocking effects were found exclusively in a single isomer. On the basis of \textit{pA}_2 values, (+)-hyoscyamine has $\frac{1}{100}$ the blocking activity of the active (−)-hyoscyamine. According to pharmacologic data, the absolute configuration of the active isomer of tropicamide must be identical with that of the active isomer of hyoscyamine, namely, 2S. It indicates that the orientation of important functional groups in either (−)-hyoscyamine or (−)-tropicamide for interaction with the receptor must be the same.

Whenever the activity difference between the isomers is large, the racemate usually possesses half the activity of the potent isomer. Occasionally it is advantageous to separate the inactive isomer from the active isomer because the contribution of the inactive isomer to the toxicity sometimes exceeds that of the active isomer. Thus the therapeutic ratio for the active isomer could be improved. Although the merit of racemic tropicamide as a mydriatic and cycloplegic is well established, the relative merit of the (−)-isomer over the racemate remains to be elucidated. Our data indicate that as compared to the (+)-isomer,
(-)-tropicamide is a more potent muscarinic blocker by a factor of 50 to 75.

Relative to the effects observed in the nonpigmented iris, tropicamide isomers are two to three times less active in the pigmented iris. These results are very similar to the blocking effects of atropine in the two types of irides. Detailed studies with atropine indicate that the drug is absorbed by the pigment cell melanin in the dark iris. The loss of atropine to the melanin granules could decrease the free concentration of the drug in the vicinity of the muscarinic receptor. Thus the apparently diminished concentration will produce lesser mydriatic effects. This factor obviously does not exist in the nonpigmented eye. The converse is expected during the wash-off studies. Relative to that observed in the nonpigmented iris, the blocking effects of (-)-tropicamide were very slowly reversed. During the wash-off, the blocker which is accumulated by the melanin granule could be slowly released to produce relatively prolonged local muscarinic block.

During this study, on several occasions, the optical rotation of the isomers was checked through the courtesy of Dr. D. D. Miller of the Division of Medicinal Chemistry of our College.

From the Division of Pharmacology, The Ohio State University College of Pharmacy, Columbus. The study was supported in part by the United States Public Health Service, National Institute of Health, Grant EX-1308, and by Alcon Laboratories, Inc., Fort Worth, Texas. Submitted for publication June 26, 1977. Reprint requests: Dr. Popat N. Patel, Division of Pharmacology, The Ohio State University College of Pharmacy, 500 W. 12th Ave., Columbus, Ohio 43210.

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REFERENCES

Intraocular pressure after optic nerve transection. DONALD M. SERAFANO AND RICHARD F. BRUBAKER.

Intraocular pressure response to systemically administered osmotic agents was studied in albino rabbits with one optic nerve transected and the fellow optic nerve left intact. There was a significant increase in intraocular pressure of both eyes following water ingestion but no significant difference in the pressure rise of the two eyes. There was a significant decrease in intraocular pressure of both eyes following glycerol ingestion but no significant difference in the pressure fall of two eyes. These results do not support the hypothesis that the optic nerve carries fibers which are part of the control system for intraocular pressure.

In 1969, Ruise and Simonsen studied a series of patients who had suffered unilateral optic nerve lesions from trauma. Eyes with traumatized optic nerves had less intraocular pressure rise following water ingestion. They postulated that the optic nerve served as a communicating pathway between the eye and an osmoreceptor in the hypothalamus.

In 1970 Krupin et al. reported an animal model for investigating the relationship between optic nerve lesions and intraocular pressure. They demonstrated that optic nerve transection reduced the intraocular pressure response to systemically administered osmotic agents. After a series of experiments they concluded that a sensitive osmoreceptor exists in the hypothalamus, which may regulate or modify the response of the eye to osmotic agents via an optic nerve pathway. Cox et al. have cited preliminary evidence that, in the rabbit, this receptor may be associated with the supraoptic nucleus.

The purpose of this study was to attempt to confirm the observation that cutting the optic nerve decreases the eye's response to osmotic agents and to determine whether such a change could be explained by local effects rather than neural feedback effects.

Methods. Intraocular pressure was measured with a floating-tip gas tonometer, the Pneumatograph. This instrument was recalibrated for the living rabbit eye. By means of the closed-stopcock method of calibration, the gas flow of the