Mechanism of Methacholine-Induced Rise in Intraocular Pressure in the Dog

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Abstract □ The mechanism of the methacholine-induced rise in intraocular pressure in the dog was studied to determine the basis of the relationship, if any, between lens thickness and intraocular pressure. The results rule out a vasodilator component in methacholine intraocular pressure elevation, and the tonometric recording of the elevation makes unlikely the involvement of methacholine stimulation of the rectus muscles of the eye. Thus, indirect evidence points to methacholine stimulation of ciliary muscle contraction with consequent thickening of the lens as responsible for its intraocular pressure-elevating effect and, hence, the ability of the response under investigation to specify the cycloplegic liability of a neurotropic antispasmodic agent.

Keyphrases □ Methacholine—mechanism of induced rise in intraocular pressure, effect of phenylephrine, dogs □ Intraocular pressure—mechanism of methacholine-induced rise, effect of phenylephrine, dogs □ Phenylephrine—effect on methacholine-induced rise in intraocular pressure, dogs □ Cholinergic agents—methacholine, mechanism of induced rise in intraocular pressure, effect of phenylephrine, dogs □ Adrenergic agents—phenylephrine, effect on methacholine-induced rise in intraocular pressure, dogs

During studies to determine the relative response of a number of parameters to intravenously administered methacholine chloride, intraocular pressure, as measured with an applanation tonometer, increased during a brief postinjection period and then decreased to normal (1). In contrast, parasympathomimetic agents in general, when applied topically to the cornea, were reported (2, 3) to lower intraocular pressure. It became apparent that the increase in intraocular pressure in response to methacholine is dose dependent and that it can be offset by the prior administration of an anticholinergic compound such as atropine if the dose of the latter is adequate. Moreover, if the dose of the anticholinergic be such as to effect a partial block of established extent of the cholinergic receptors, the magnitude of the dose of methacholine that will elicit a 50% increase (half of the maximal response) in intraocular pressure (Me_{50}) is an index of the affinity of the anticholinergic agent for cholinergic receptors associated with the response.

EXPERIMENTAL

Mongrel, male dogs, 12–17 kg, were fed once a day at approximately the same time each day and allowed water *ad libitum*. Each dog was anesthetized with 10 mg of thiopental sodium/kg and 90 mg of α-chloralose/kg iv, mixed in the calculated amounts and heated in an aqueous solution to 40–45° to increase solubility just prior to injection.

Blood pressure was recorded directly by femoral artery puncture and pressure transducer. Changes in intraocular pressure during a determination were transmitted to a pressure preamplifier by an applanation pressure transducer, the pressure-sensitive metallic core of which was positioned directly over the pupil on the corneal surface. A short piece of glass tubing, flanged at its point of contact with the eyeball, was used to support the base of the tonometer so that the core of the tapered end made unobstructed contact with the corneal surface.

The dosage regimen employed in the initial portion of this study was identical with that used previously (1). The first standard dose of 5.55 μg of methacholine/kg iv was injected and subsequently flushed into the circulatory system of the animal with 3 ml of saline. Intraocular pressure responses to methacholine were read at their respective peaks. Blood pressure responses were read twice for each dose of methacholine administered. The first reading was made just prior to drug administration, and the second was made at the nadir of the depressor response.

The second standard dose of methacholine (5.55 μg/kg) was administered in the same manner as the first. If the response to either of these two drug administrations differed by much more than 10% from the average of the two, a third standard dose of methacholine was given. Then the three responses were averaged for use in subsequent calculations to determine the degree of inhibition of the response occasioned by the anticholinergic agent.

When the effect of the last preanticholinergic dose of methacholine had terminated, a 6.5-μg/kg iv dose of the anticholinergic agent, glycopyrrolate bromide, was administered. During the following 30 min, the anticholinergic compound was allowed to exert its full effect. Then the preanticholinergic dose of methacholine (5.55 μg/kg) was repeated, and the responses of the two parameters to this dose were read in the same manner as described previously. After ensuring that all records had returned to their respective baselines, a second dose of methacholine was administered 15 min after the administration of the initial postanticholinergic dose; similarly, a third dose of this drug was administered 15 min following the second dose.

In all determinations, the quantities of the postanticholinergic doses of methacholine were equivalent to: (a) the preanticholinergic dose, (b) twice the preanticholinergic dose, and (c) three times the preanticholinergic dose. This series was chosen in an attempt to elicit responses such that one would be approximately 50% of the (average) preanticholinergic response, one would be greater than 50% of this (average) response, and one would be less than 50%.

Each postanticholinergic response to methacholine was expressed as the percent inhibition of the (average) preanticholinergic response. An Me_{50} was then obtained for each parameter whose responses lent themselves to quantitative treatment (intraocular pressure and arterial blood pressure) by plotting dose–response data on logarithmic–probability paper, with the percent inhibition as the ordinate and the dose of methacholine as the abscissa (single dose of anticholinergic per determination). The fit of the line to the plotted points was determined both by visual inspection and by the method of least squares. The Me_{50}'s so obtained were averaged, and their standard deviations were determined.

The first modification of the described drug dosage regimen was the substitution of an intravenous dose of phenylephrine hydrochloride for glycopyrrolate. Doses of 10, 20, and 30 μg/kg of the latter were administered 15 min apart, beginning 15 min after the last prephenylephrine dose of methacholine had been injected. Responses were recorded in the usual manner.

Exactly 60 sec after the injection of the first dose of phenylephrine, at the peak of the response to this pressor agent, a dose of methacholine equivalent to the prephenylephrine dose (5.55 μg/kg) was administered and the responses were recorded. After both intraocular pressure and blood pressure parameters had again returned to their respective baselines, the second phenylephrine dose was administered, followed 1 min later by a dose of methacholine identical to the one previously administered. The third and largest dose of phenylephrine was then given, again followed precisely 1 min later by 5.55 μg/kg of methacholine. By means of the prior systemic administration of phenylephrine, a potent vasoconstrictor and pressor agent, it was possible to obtain pharmacological opposition to the depressor and vasodilator effects of methacholine which might be involved in the intraocular pressure rise.

RESULTS AND DISCUSSION

Variations in such physiological phenomena as respiration and arterial blood pressure can affect intraocular pressure (4), and intraocular pressure elevated as a result of such variations has been reported. Carballo (4) stated that acetylcholine and anticholinesterases elevate ocular tension by producing vasodilation in the eye and by stimulating the contraction of the external rectus muscles. This finding was substantiated

Table I—Methacholine-Induced Responses of Intraocular Pressure and Arterial Blood Pressure after Injection of Phenylephrine Hydrochloride in a 16.0-kg Dog

Time	Drug	Pupil Size, mm	Intraocular Pressure, mm Hg	Arterial Blood Pressure, mm Hg
2:45	Thiopental, 10 mg/kg iv, and chloralose, 90 mg/kg iv	—	—	—
3:00	Chloralose, 45 mg/kg sc	—	—	—
3:10		3	—	170/105
3:20		3	—	182/115
3:22	Methacholine, 55 μg/kg iv	4	13.0	70/50
3:32		3	—	198/120
3:37	Phenylephrine, 10 μg/kg iv	4	—	240/180
3:38	Methacholine, 5.55 μg/kg iv	4	14.0	150/80
3:48		3	—	200/122
3:53	Phenylephrine, 20 μg/kg iv	4	—	250/180
3:54	Methacholine, 5.55 μg/kg iv	3	12.5	182/120
4:04		4	—	200/130
4:09	Phenylephrine, 30 μg/kg iv	4	—	250/90
4:10	Methacholine, 5.55 μg/kg iv	4	13.0	170/120

by Colle *et al.* (5), who observed that administration of large doses of acetylcholine produced a rapid rise in intraocular pressure of the perfused dog head, with a rise in systemic blood pressure. Here also vasodilation of intraocular vessels and contraction of the rectus muscle were implicated in the response. Methacholine, certain anticholinesterases, and pilocarpine have all caused a vasodilation of the conjunctival and intraocular vessels as well as increased permeability of their walls (6). The volume of blood contained in the vessels of the choroid and of the ciliary body constitutes a large part of the total volume of the eye. Hence, any agent affecting the filling of these vessels may affect intraocular pressure (7).

Cholinergic agents, especially echothiophate iodide and pilocarpine, may produce a shallowing of the anterior chamber, which could result from an anterior displacement of the lens with relaxation of the suspensory ligaments following contraction of the ciliary muscle (8). The thrust of the anterior lenticular displacement may cause a bowing of the

↑ PREPHENYLEPHRINE RESPONSES

↑ RESPONSES AFTER 10 μg/kg

↑ RESPONSES AFTER 20 μg/kg

↑ RESPONSES AFTER 30 μg/kg

Figure 1—*Intraocular and arterial blood pressure responses to methacholine chloride (5.55 μg/kg) in an anesthetized dog before and after injection of 10, 20, and 30 μg of phenylephrine hydrochloride/kg iv. (Time: 1 mm = 1 sec; arterial blood pressure: 1 mm = 10 mm Hg; intraocular pressure: 1 mm = 1 mm Hg.)*

iris in the direction of the cornea, narrowing the chamber angle and increasing resistance to drainage. As the thickening lens pushes anteriorly against the iris, its more spherical anterior surface may achieve a pupil block, which prevents dissipation of a pressure rise through this avenue of escape.

Thus, there are three (chief) mechanisms by which parasympathetic stimulation and parasympathomimetic agents may increase intraocular pressure such as is obtained in the intravenous administration of methacholine chloride in the dog:

1. Increased aqueous humor production through vasodilation and increased permeability of ciliary vessels.
2. Anterior thickening of the crystalline lens pushing the iris toward the rigid cornea, thereby decreasing the drainage angle of the anterior chamber and the chamber volume. (A combination of 1 and 2 is possible.)
3. Contraction of the external rectus muscle with consequent reduction in intrachamber volume.

In light of results obtained when phenylephrine was injected intravenously, vasodilation probably does not play a significant role in the methacholine-induced rise in intraocular pressure in the intact dog (Table I and Fig. 1). Little, if any, change occurred in the elevation of intraocular pressure after methacholine injection when phenylephrine was administered intravenously beforehand and was acting systemically to constrict intraocular vessels or was applied topically, directly upon the corneal surface (not shown). Phenylephrine was used to eliminate the possibility that intraocular vessel changes elicited by methacholine are responsible for the rise in intraocular pressure. This sympathomimetic mydriatic agent is reported to lower significantly an elevated intraocular pressure by decreasing aqueous humor formation through constriction of the intraocular vessels of the ciliary body and the choroid coat. Furthermore, it induces no significant changes in the aqueous humor outflow, the ciliary muscle, or the crystalline lens (9-11).

A comparison of the mean and standard deviation of the mean blood pressure Me_{50} values with the mean and standard deviation of the intraocular pressure Me_{50} values for glycopyrrolate shows a significant difference ($p = 0.005$) between the affinities of vascular and ciliary cholinergic receptors for this anticholinergic agent (Table II). This difference indicates that the rise in intraocular pressure elicited by methacholine is not secondary to the fall in arterial blood pressure, *i.e.*, to vasodilation and the subsequent increase in aqueous humor production. Thus, the two responses do not appear to represent the same basic phenomenon and might be separable on an experimental basis. Moreover, the rapidity of the rise of intraocular pressure in response to methacholine makes increased aqueous humor production an unlikely etiologic factor. Besides, the contraction of ciliary muscle in response to methacholine reduces muscle volume, with a resultant constriction of ciliary blood vessels, each of which tends to lower intraocular pressure. On the other hand, the reduction in the diameter of the ciliary circle, which attends contraction of the ciliary muscle, itself reduces the size of the anterior chamber and favors a pressure rise. In the early work of Armaly (8), the latter effect predominated in the cat eye after parasympathetic stimulation.

Because of the nature of the intraocular pressure determination (employing an applanation tonometer), any contraction of the external rectus muscle would manifest itself as a decrease in intraocular pressure and would be thus detectable.

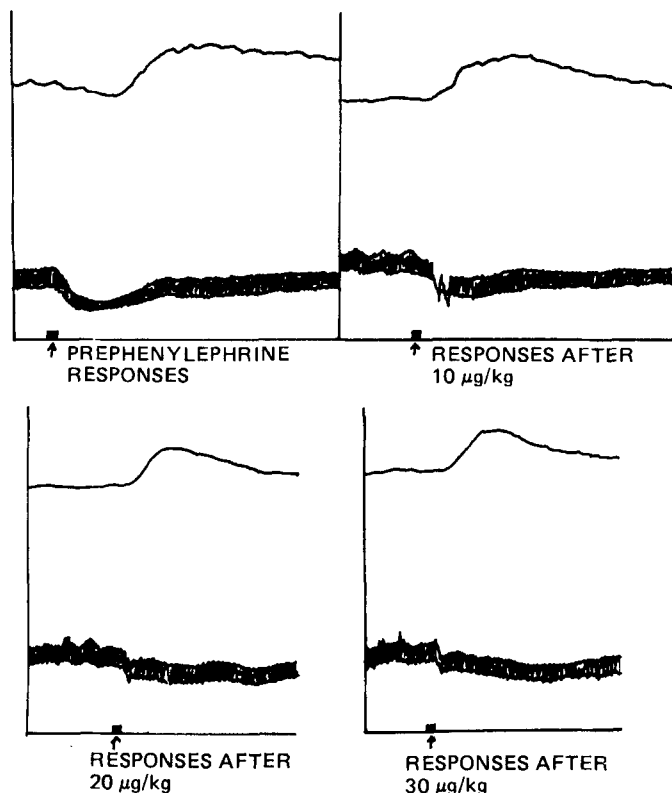By deduction, then, it appears that a thickening of the crystalline lens

Table II—Determination of Me_{50} Values for Glycopyrrolate (6.5 μg/kg iv)

	Intraocular Pressure, mm Hg	Inhibition, %	Arterial Blood Pressure, mm Hg	Inhibition, %
Mean preantispasmodic	12.0		85.00	
Methacholine, 5.55 μg/kg iv	4.0	66.66	35.02	58.80
Methacholine, 11.10 μg/kg iv	6.0	50.00	49.98	41.20
Methacholine, 16.65 μg/kg iv	7.5	37.50	57.46	32.40
Me_{50} value (from graphs)	10.08 μg/kg			8.10 μg/kg
Me_{50} mean	9.95 + 2.14 $(n = 9)$			5.79 ± 1.61 $(n = 16)$
$t = 5.489$				

with a resultant thrust of the iris in the direction of the cornea, decreasing the chamber volume and drainage angle, together with a (possible) block of the pupillary opening preventing the dissipation of increasing chamber pressure is responsible for the methacholine-induced rise in intraocular pressure. This rise probably occurs only after intravenous injection of methacholine when the rapid forward thrust of the iris by the thickening of the lens, which appears responsible for it, is able to overcome for a time the tendency of the drainage system to offset it, a tendency soon realized in the speedy return of pressure to baseline following the postinjection rise. Subsequent investigation might involve another route of methacholine administration in an effort to determine the influence of the administration route on the response. It should also attempt to determine whether a dose of methacholine that is threshold for blood pressure reduction has any effect on intraocular pressure or, conversely, the extent to which methacholine might reduce blood pressure before it effects an increase in intraocular pressure.

The significance of this study involves the use of the parameter of intraocular pressure to determine the liability of a neurotropic antispasmodic (parasympatholytic) agent to induce cycloplegia. If a direct relationship exists between intraocular pressure and lens thickness, as this study indicates, then the extent to which the neurotropic antispasmodic interferes with a cholinergic-induced rise in intraocular pressure is a measure of its tendency to produce cycloplegia or blurring of vision. Hence, neurotropic antispasmodic compounds with relatively high intraocular pressure Me_{50}'s (in relation to their antimotility or antisecretory Me_{50}'s) should have a high liability with respect to the side effect of cycloplegia.

The study reported is a facet of a comprehensive investigation conducted over years. Numerous determinations of the effect of neurotropic antispasmodic agents on the intraocular pressure response to methacholine in the dog were made, and the data reported here are representative.

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Impurities in Drugs I:
Imipramine, Desipramine, and Their Formulations

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Abstract □ Nineteen lots of imipramine tablets and four lots of desipramine tablets were examined for impurities by TLC. Iminodibenzyl, desipramine, and 10,11-dihydro-5-[3-(methylamino-3'-dimethylaminopropyl)propyl]-5H-dibenz[b,f]azepine dihydrobromide (I) were found in some imipramine tablets, and iminodibenzyl and imipramine were found in some desipramine tablets, all at levels of less than 0.3% of label claim of the drug. Except for I, the identity of the impurities was established by comparison with known standards; I was synthesized and its composition was established by elemental analysis. All impurities, including I, were characterized by TLC, GLC, and mass spectrometry.

Keyphrases □ Imipramine tablets—impurities, TLC, GLC, and mass spectral analysis □ Desipramine tablets—impurities, TLC, GLC, and mass spectral analysis □ Impurities—in imipramine and desipramine tablets, TLC, GLC, and mass spectral analysis □ Tablets—imipramine and desipramine, TLC, GLC, and mass spectral analysis of impurities □ Dosage forms—tablets, imipramine and desipramine, TLC, GLC, and mass spectral analysis of impurities □ Antidepressants—imipramine and desipramine tablets, TLC, GLC, and mass spectral analysis of impurities

Organic compounds found as impurities in drugs and drug formulations may be intermediates or by-products of the drug synthesis, products of drug or excipient degradation, products of drug–excipient interaction, or the result of contamination. The nature of the impurities in a drug may depend on the synthetic process and the source of materials used in manufacture or the nature and source of the excipients. Impurities may be toxic, and their