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REFERENCES


Subsensitivity to pilocarpine in primate ciliary muscle following topical anticholinesterase treatment. PAUL L. KAUFMAN AND EBNST H. BARANY.

Accommodative responses to intramuscular pilocarpine were determined in four surgically amnestic ververt monkeys, before and after eight weeks of daily unilateral topical treatment with echothiophate iodide. The echothiophate-treated eyes maintained maximum myopia during the treatment course. However, after echothiophate treatment was stopped and the refraction had returned to baseline, a subsensitivity of the accommodative mechanism to pilocarpine became apparent. Normal sensitivity to pilocarpine did not return until four to five months after echothiophate treatment had been stopped.

Subacute or chronic systemic treatment with anticholinesterase (ChE) agents causes various non-ocular cholinergic end-organ systems to become subsensitive to direct acting cholinomimetics.1,2 Topical anti-ChE treatment induces subsensitivity to cholinergics in the irides of several mammalian species.2,3 We report here the occurrence of this phenomenon in primate ciliary muscle.

Materials and methods.
Animals. Four adult female vervet monkeys (Cercopithecus ethiops) weighing 2.5 to 3.5 kilograms had both irides totally removed to facilitate objective refraction (PLK, unpublished technique). The anterior chambers were free of cells and flare and the lenses crystal clear on slit lamp examination at the start of the experiments two months postoperatively.

Refraction. The animals were anesthetized with intramuscular sodium methohexitol (Brietal, Lilly) 15 mg. per kilogram (refraction only) or methohexitol followed by intramuscular pentobarbital 30 to 35 mg. per kilogram (refraction under systemic pilocarpine). Refraction was performed with a Thorner refractometer.7 To improve the optics and expand the minus range of the refractometer, methylmethacrylate contact lenses of known minus power were placed on the corneas.

Pilocarpine testing. Deep intramuscular injections of pilocarpine-HCl solution were given in the thigh. Each eye was then refracted every four to eight minutes until a stable myopia was reached and began to fade. Maximum myopia was taken as the mean of at least two successive determinations on this plateau. Each animal received pilocarpine-HCl doses of 0.1, 0.2, 0.5, 1.0, 2.0, and 3.0 mg. per kilogram.7 At each session, the baseline refraction was determined, and one or two doses of pilocarpine were then administered. When two doses were given, the sequence was: 0.1 followed by 1.0, or 0.2 followed by 2.0, or 0.5 followed by 3.0. The second dose was not given until the effect of the first began to fade (generally 30 to 40 minutes separated the doses). Accordingly, the first dose was ignored, i.e., the second dose in the session, 0.2, 2.0, was considered to be 2.0 rather than 2.2 mg. per kilogram, etc. The pharynx was suctioned intermittently. At the end of a session, atropine sulfate 0.01 mg. (salt) per kilogram was injected intramuscularly. At least 48 hours separated the sessions, insuring that the effect of the atropine had disappeared completely (PLK, unpublished observations). The system, consisting of experimenter, refractometer, animal, and drug gave reproducible results. For instance, monkey No. 1 was given intramuscular pilocarpine, 1.0 mg. per kilogram, three times before the start of topical treatment, and showed very similar responses in each eye on all three occasions (Fig. 1).

Anti-ChE treatment. Echothiophate iodide 0.25 per cent solution (PI), as commercially available eye drops (Phospholine iodide 0.25 per cent, Ayerst) was used. A control solution, identical except for the absence of echothiophate iodide was prepared (diluent). The monkeys were treated twice daily (approximately 9 A.M. and 9 P.M.) on weekdays and once daily on weekends. Never did more than 24 hours elapse between treatments. One eye of each monkey was treated with PI, the other eye with diluent. Separate droppers, each specially made to deliver only 4 µl per drop, were used for the two solutions. Two monkeys had PI applied to the right eye only.
eye, and two received PI in the left eye. For daytime treatments, the monkeys were trained to jump from their cages into a net in which they were restrained supine by an assistant. The lids were retracted digitally and four drops of either PI or diluent were placed on the central cornea at intervals of 30 seconds. Blinking was prevented between drops and for 30 seconds after the last drop. The lid margins were then swabbed with cotton. For evening and weekend treatments, the monkeys were anesthetized with methohexital, placed supine on a table, and a lid speculum was inserted. Different specula were used for eyes receiving PI or diluent. Four drops of either solution were administered at 30-second intervals. The lid speculum was removed 30 seconds or more after the last drop and the lid margins swabbed with cotton. The refraction was measured every few days before the drops were administered.

Slit lamp examination was performed periodically under methohexital anesthesia.

**Results.** Fig. 1 shows the refractions for each animal over the entire course of the experiment. For the first 2.5 weeks of topical treatment the refraction was estimated by direct ophthalmoscopy; however, it was difficult to obtain precise readings in the strongly accommodated eyes. Therefore, we returned to the more cumbersome but more reliable refractometer. Monkeys Nos. 1, 3, and 4 clearly maintained myopia equal to the maximum previously induced by intramuscular pilocarpine until the end of the eight weeks of PI treatments. Monkey No. 2 may have shown a slight decrease over the last four weeks of treatment. The few refractions performed on each animal 21 to 24 hours after PI treatment revealed essentially the same myopia as those performed 12 hours after treatment.

Monkey No. 4 aspirated and died the day after treatment was stopped while being anesthetized for slit lamp examination. The myopia in the PI-treated eyes of the three remaining monkeys gradually decreased to near pretreatment baseline levels over the next three weeks. The refraction in the diluent-treated eyes did not appear to change during either the treatment or the recovery periods. Nine and one-half weeks after topical treatment had been discontinued, the monkeys were given intramuscular pilocarpine-HCl, 1.0 mg. per kilogram, which had previously caused strong but submaximal accommodation. The baseline refractions were essentially unchanged from their pretreatment values, and the diluent-treated eyes showed the same accommodative response as previously. However, the PI-treated eyes accommodated much less (Fig. 1, A through C). Accommodation in the PI-treated eyes was 6.21 ± 0.30 (mean diopters ± S.E.), which was significantly less than in the diluent-treated opposite eyes (13.25 ± 2.01, p < 0.05 by the two-tailed Student t-test), and significantly less than in the PI-treated (13.26 ± 1.64, p < 0.02) and diluent-treated (13.00 ± 1.98, p <
PILOCARPINE (mg/kg)

BEFORE AFTER

DILUENT TREATED PI TREATED

Fig. 2. Accommodation induced by intramuscular pilocarpine HCl, 0.1 to 3.0 mg per kilogram before eight weeks of topical PI (echothiophate) treatment and during the twelfth week after treatment was stopped. A through C: monkeys 1 through 3, respectively. For monkey No. 3, the 9.5-week post-treatment response to the 1.0 mg per kilogram dose was used (see legend for Fig. 2). Solid circles, solid line = pretreatment accommodation expressed as percentage of pretreatment maximum; solid circles, dashed line = post-treatment accommodation expressed as percentage of pretreatment maximum; open circles, dashed line = post-treatment accommodation expressed as percentage of post-treatment maximum. Standard errors of the geometric mean doses are shown by the horizontal bars, and are sometimes smaller than the points representing the doses.

Accommodative responses to intramuscular pilocarpine, 0.1 to 3.0 mg per kilogram, were then determined during the twelfth week after stopping treatment (Fig. 2). In each animal, the responses of the diluent-treated eye before and after treatment and the PI-treated eye before treatment were quite similar. However, the responses of the PI-treated eye after treatment to submaximal pilocarpine doses were markedly less. It could not be excluded that the maximum accommodation had diminished slightly in the PI-treated eyes. The pilocarpine doses were prefixed, and in two animals plateau was not quite reached at the highest dose (3.0 mg per kilogram). The major change, however, was clearly a shift of the dose-response curves to the right, i.e., a subsensitivity.

Geometric mean dose-response curves for the PI-treated eyes before treatment and 11 to 12 same animal by 50.2 ± 9.4 per cent (mean ± S.E., p < 0.05 by the two-tailed paired t-test) and less than that in the PI-treated eye of the same animal before treatment by 51.3 ± 7.5 per cent (p < 0.025).

Accommodative responses to intramuscular pilocarpine, 0.1 to 3.0 mg per kilogram, were then determined during the twelfth week after stopping treatment (Fig. 2). In each animal, the responses of the diluent-treated eye before and after treatment and the PI-treated eye before treatment were quite similar. However, the responses of the PI-treated eye after treatment to submaximal pilocarpine doses were markedly less. It could not be excluded that the maximum accommodation had diminished slightly in the PI-treated eyes. The pilocarpine doses were prefixed, and in two animals plateau was not quite reached at the highest dose (3.0 mg per kilogram). The major change, however, was clearly a shift of the dose-response curves to the right, i.e., a subsensitivity.

Geometric mean dose-response curves for the PI-treated eyes before treatment and 11 to 12
weeks after stopping treatment were constructed (Fig. 3). Because of differences among animals in maximal diopters of accommodation, accommodation was expressed as a percentage of maximum. Pretreatment accommodation is shown as a percentage of pretreatment maximum. Post-treatment accommodation is shown as a percentage of both pre- and post-treatment maximum; the former may slightly overestimate the dose needed for a given response while the latter may slightly underestimate it. Eleven to twelve weeks after stopping PI treatment, nearly twice the pretreatment dose was needed to achieve a given response, i.e., the loss of sensitivity was nearly 50 per cent.

The animals were then given intramuscular pilocarpine, 1.0 mg. per kilogram, periodically to see how long the subsensitivity would persist (Fig. 4). The accommodative response in the PI-treated eyes increased very gradually in two animals and relatively abruptly in one, reaching pretreatment levels 16 to 22 weeks after the cessation of treatment. The responses in the diluent-treated opposite eyes varied little from the pretreatment values.

No animal evidenced systemic cholinergic toxicity and no eye evidenced anterior segment reaction (i.e., conjunctival hyperemia, aqueous cells, or flare) at the slit lamp at any time. All the PI-treated eyes developed subcapsular lens opacities; none of the diluent-treated eyes did. The lens findings will be reported elsewhere.

Discussion. While other mechanisms cannot be excluded, our findings are compatible with the following three concepts: (1) the sensitivity of cholinergic end organs to endogenous acetylcholine (ACh) and exogenous cholinomimetics depends inversely on the average level of exposure to the neurotransmitter. (2) all the receptors for an agonist need not necessarily be occupied to obtain a maximal tissue response to the agonist. The tissue may have "spare receptors" or a "receptor reserve" for the agonist. (3) the ciliary muscle may be capable of contraction in excess of that required to produce maximal accommodation ("contraction reserve"), although this point is controversial.

The excess ACh present as a result of PI treatment would cause a decrease in sensitivity of the ciliary muscle to ACh and other cholinomimetics which act on the same receptor, perhaps, as suggested by Bito and co-workers, by causing a decrease in receptor concentration on the effector cell membrane. The presence of a receptor reserve and/or a contraction reserve would still permit maximum accommodation if the local concentration of agonist (ACh or pilocarpine) were high enough to overcome the subsensitivity. The subsensitivity would be apparent only at submaximal doses of agonist.

At present we cannot explain the persistence of the induced subsensitivity for so long after PI treatment had ended.

We do not know whether our findings, or those of Bito and co-workers, have counterparts in the human eye. That induced miosis and myopia do not disappear during anti-ChE treatment does not prove the absence of cholinergic subsensitivity (see above). The phenomenon has not been searched for in a systematic pharmacological manner in the human eye.
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Key words: acetylcholine, cataract, cholinergic subsensitivity, cholinesterase inhibitors, ciliary accommodation, ocular refraction, pilocarpine.

REFERENCES

The inhibiting effect of indomethacin on the disruption of the blood-aqueous barrier in the rabbit eye. Elisabeth Bengtsson.

The aqueous flare (AF) of an intact rabbit eye was measured by a photoelectric instrument. Local application of prostaglandin E, (PGE,) and its precursor arachidonic acid (AA) gave an almost identical increase of the AF. The response to AA but not to PGE, was inhibited by pretreating the eye locally with a solution of indomethacin. The ability of indomethacin to inhibit the aqueous flare response (AFR) to an agent is assumed to indicate that a kind of prostaglandin is the effecter of the AF. Indomethacin blocked the AFR to infrared irradiation of the iris and to intravenous administration of endotoxin but not to subcutaneous administration of &alpha;-melanocyte-stimulating hormone (&alpha;-MSH).

The rabbit eye responds to both mechanical and chemical traumata with miosis, local ocular vasodilation, a sustained rise in intraocular pressure, and an increased capillary permeability, seen as a breakdown of the blood-aqueous barrier resulting in a marked rise in the protein content of the aqueous humor. This was first demonstrated by Wessely in 1908, and has since been repeatedly confirmed. This inflammatory reaction can be mimicked by locally applied prostaglandins (PG's), which can be formed by the tissues of the iris and the ciliary body. Some systemically administered substances can also cause a breakdown of the blood-aqueous barrier, resulting in an aqueous flare response (AFR). Thus, certain peptides of pituitary origin are known to yield an AFR when given subcutaneously to rabbits. The AF-producing ability of the different amino acid chains closely follows their melanocyte stimulating activity, whereas, it seems to be independent of their corticotropic activity. Finally, endotoxins of different bacteria give an AFR when administered intravenously.

Since traumata of different kinds as well as topically and systemically administered drugs are able to provoke very similar effects, it does not seem unlikely that there is a common factor responsible for the final step. In fact, Beitch and Eakins in 1969 attributed this role to prostaglandins. It is known that indomethacin and aspirin-like drugs inhibit the conversion of PGE, and PGE, from their precursor, arachidonic acid (AA). It has also been shown that aspirin reduces the increase of protein in aqueous humor after paracentesis and argon laser radiation of the iris indicating that these effects are mediated by...