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SUBSENSITIVITY TO CHOLINOCEPTOR STIMULATION OF THE HUMAN IRIS SPHINCTER *in situ* FOLLOWING ACUTE AND CHRONIC ADMINISTRATION OF CHOLINOMIMETIC MIOTIC DRUGS

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1 Maximal pupillary miosis was obtained with single topical applications of 4 cholinomimetic drugs in therapeutic concentrations to normal human subjects.

2 When the pupil had recovered from the miosis, there remained a reduced light reflex response of 22.7% at 24 h after aceclidine, 18.0% at 31 h after pilocarpine, 10.3% at 48 h after physostigmine and 4.9% at 7 h after arecoline.

3 This reduced sensitivity to light was accompanied by an overshoot of the resting pupil diameter and, after aceclidine miosis, a reduced response to a second application of miotic.

4 Similar findings were observed in glaucoma patients following withdrawal of chronic pilocarpine therapy.

5 It is suggested that the slowly reversible after-effects of acute and chronic administration of cholinomimetic miotics can be explained by desensitization of iris sphincter cholinoceptors.

Introduction

The size of the pupillary reflex response to a standard light stimulus has been shown to decline in proportion to the degree of miosis resulting from application of cholinoceptor agonists to the eye (Smith & Smith, 1978). This proportional relationship, which was independent of the particular miotic used, illustrates a general principle whereby the response to a fixed stimulus is reduced by raising the baseline (compare, for example, the pressor response to intravenous noradrenaline in the rat). When the recovery from pilocarpine miosis was followed, however, the light reflex returned to normal more slowly than did resting pupil size so that a 20% to 30% reduction persisted when no miosis remained (Newsome & Loewenfeld, 1974; Smith, Smith & Lazare, 1978). In the present study other cholinomimetic drugs have been tested for a similar after-effect on both sensitivity to light and to a second application of drug. Further, evidence of iris sphincter subsensitivity has been sought during withdrawal of chronic pilocarpine therapy from glaucoma patients.

Methods

Studies on normal volunteers

The following drugs were applied topically at 2 week intervals to one eye of each of 7 healthy subjects aged 22 to 27 years: 2 drops of pilocarpine (Smith & Nephew) 2% given 1 min apart; 10 drops of aceclidine hydrochloride (Chibret) 0.2% administered over 30 min; 10 drops of arecoline hydrobromide (Sigma) 0.2% administered over 20 min; 1 drop of physostigmine sulphate (T. & H. Smith) 0.5%; 5 drops of saline (0.9% w/v NaCl solution) given 1 min apart. Pilocarpine, aceclidine and physostigmine were given in therapeutic doses which, as with the arecoline dose, gave maximal pupillary constriction (Smith & Smith, 1978). Only 4 of the 7 subjects participated in the physostigmine experiments.

Vertical pupil diameters were measured at rest (in darkness) and during reflex responses to open-loop light stimulation by television pupillometry as described previously (Smith, Smith & Lazare, 1978). Responses were elicited in both eyes by light flashes directed at the untreated eye. Measurements were performed before and at regular intervals for up to 4 days following drug administration to establish peak effects and the time course of recovery.

A second series of experiments was designed to test iris sphincter sensitivity to a second application of miotic during the after-effect of aceclidine treatment. Arecoline was used since it has little after-effect itself (see results). Sensitivity to arecoline was measured in a further 7 subjects before, during and following the after-effects of aceclidine. The arecoline concentration that gave near 50% maximum miosis was determined

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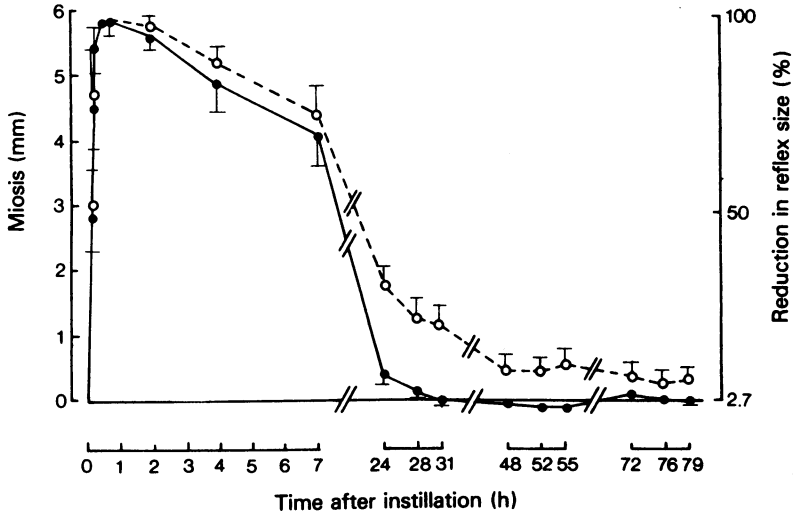

Figure 1 The miosis (●) and reduction in reflex size (○) resulting from topical pilocarpine application at zero time. Points and vertical bars represent the means and s.e. means from 7 subjects. Before drug treatment the mean reflex amplitude of the pupil to be treated was 2.7% smaller than that of the control pupil.

individually in preliminary experiments. Ten drops of this concentration were applied to both eyes at intervals of 1 min and the peak miosis recorded. The experiment was repeated a week later during the after-effect from aceclidine applied to the right eye 24 to 28 h previously, and again 2 weeks later when residual effects of aceclidine on light reflex responsiveness had disappeared.

Studies on glaucoma patients

The recovery of pupil size and light reflex amplitude was measured for 1 week during withdrawal of pilocarpine eye drop therapy in 7 patients (aged 51 to 70 years) with bilateral chronic simple glaucoma. Treatment was continued with oral acetazolamide (Diamox Sustets 500 mg twice daily). On the first day of the study the patients were given their final application of drops and the effects on pupil diameter and reflex amplitude were measured 40 min later as above. The measurements were repeated 1, 2, 4 and 7 days after withdrawal of pilocarpine.

Where appropriate, results are expressed as means ± s.e.

Results

Studies on normal volunteers

The 4 cholinomimetic miotic drugs produced maximum miosis and complete inhibition of the light reflex within 1 h after instillation of the first drop (Table 1). Miosis was calculated as the difference in resting diameter between treated and untreated eyes corrected for any physiological anisocoria, and drug effects on the light reflex were expressed as the left-right difference in reflex amplitude calculated as a percentage of that of the untreated eye. There was no change in pupillary measurements made at regular intervals for 3 days following saline application.

In the absence of drug effects the pupillary reflex amplitude of the control eye at which the light stimulus was directed exceeded that of the eye to be treated in 5 of the 7 subjects. This represented normal contraction anisocoria described elsewhere (Smith, Ellis & Smith, 1979) and appears in Figures 1 to 3 as a 2.7% mean reflex reduction present at zero time.

Following pilocarpine (Figure 1) the miotic and

Table 1 Peak pupillary effects of four cholinomimetic miotic drugs in 7 subjects

	Peak miosis (mm)	*Peak reflex reduction (%)*
Pilocarpine	5.82 ± 0.20	99.3 ± 0.7
Aceclidine	5.89 ± 0.20	100
Arecoline	5.64 ± 0.22	98.3 ± 1.7
Physostigmine	5.72 ± 0.18	100

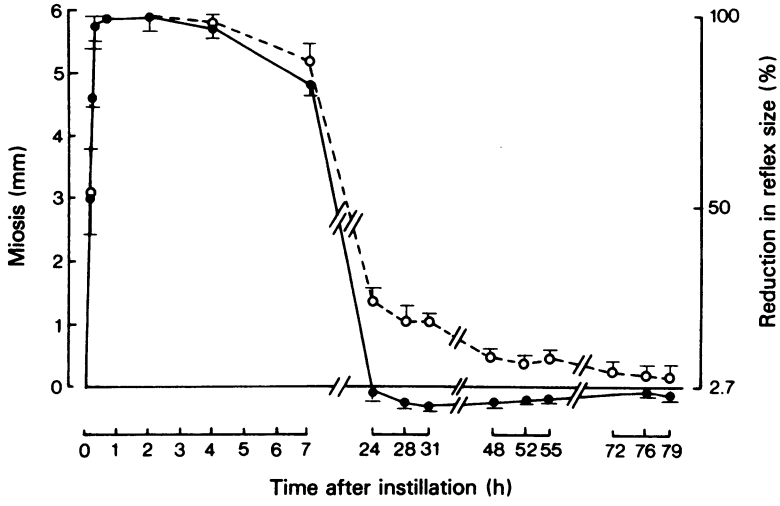

Figure 2 Pupillary effects in 7 subjects of aceclidine eyedrops, the first of which was given at zero time. Symbols as in Figure 1.

reflex effects recovered at different rates so that when the resting diameter had regained its normal size at 31 h after treatment, there remained a mean reflex reduction 18% greater than before treatment. This declined slowly over the following 48 h. During this period the pupillary measurements in darkness showed that the treated pupil had become slightly larger than the control in most of the subjects. The recovery from aceclidine treatment (Figure 2) was similar to pilocarpine in that at 24 h after treatment a 22.7% mean reduction in reflex amplitude persisted when no miosis remained. Thereafter in all subjects the residual reduction was accompanied by slight mydriasis.

The after-effect from arecoline treatment (Figure 3) was less obvious than with the other 3 drugs. The miosis had worn off completely after 7 h when a 4.9% mean reflex reduction remained. There was no after-effect apparent by 48 h after treatment.

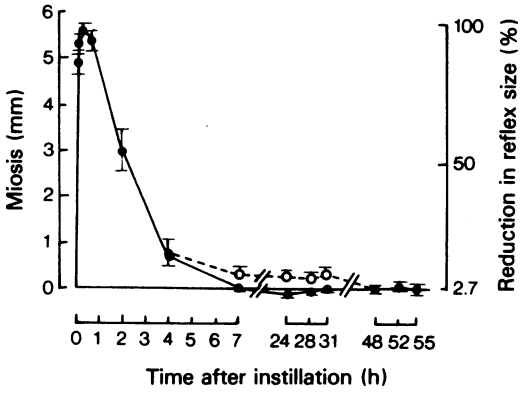

Figure 3 Pupillary effects in 7 subjects of arecoline eyedrops, the first of which was given at zero time. Symbols as in Figure 1.

The effect of physostigmine on light reflex amplitude (Figure 4) was more complex than that of the 3 directly acting cholinoceptor agonists. During the onset of action the reflex was potentiated so that at 10 min after drug application the mean miosis of 0.80 mm was accompanied by an increase in reflex amplitude of 21.7%. Thereafter the light reflex was reduced in amplitude. The reduction per mm of miosis was less than with the other 3 drugs except when the pupil diameter had reached its physical minimum at the peak of drug action. Complete recovery from the miosis did not occur until 48 h after treatment and it was accompanied by a residual reduction of 10.3% in reflex size. An after-effect of mydriasis with reflex reduction followed.

During the period of after-effect of aceclidine miosis the response of the pupil to a second application of miotic was reduced (Table 2). Before aceclidine treatment there was no significant difference between the pupils in arecoline sensitivity (mean L minus R difference in miosis was 0.08 ± 0.10 mm, $n = 7$). On repeating the arecoline test 24 to 28 h after aceclidine treatment of the right eye as above (Figure 2), the right pupil constricted significantly less than the left (by 0.71 ± 0.15 mm, $n = 7$, $P < 0.005$). Expressed as a percentage of the response of the control pupil, the mean reduction in arecoline sensitivity was $20.2 \pm 4.4\%$ ($n = 7$). This reduction was of similar magnitude to that affecting the light reflex (see above). The third arecoline experiment performed 2 weeks later showed that in six of the seven subjects tested the decreased sensitivity was reversible (mean L

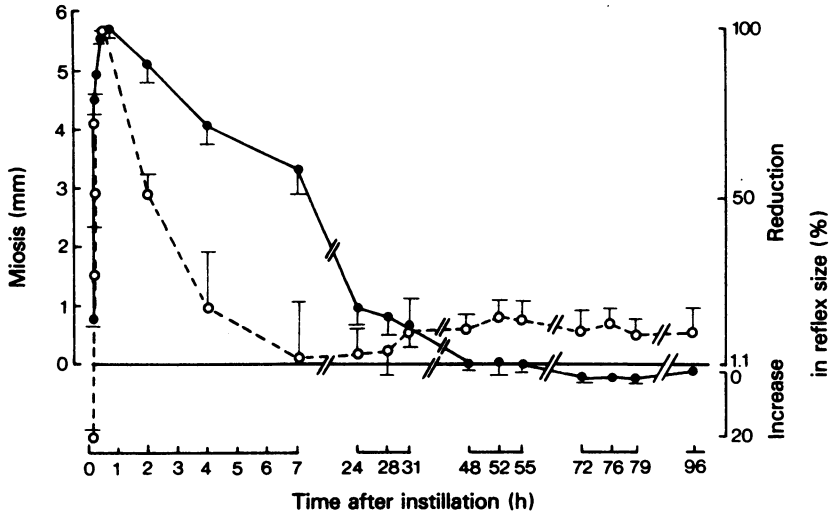

Figure 4 Pupillary effects of topical physostigmine given at zero time. The reflex amplitude of the treated eye was at first increased and then reduced relative to that of the control pupil. Shown are the means and s.e. means from 4 subjects. Before drug application the mean reflex amplitude of the pupil to be treated was 1.1% smaller than that of the control pupil.

Table 2 Response of the left (L) and right (R) pupils to topical arecoline (A) before (B) during and (C) following the after-effect from aceclidine treatment of the right eye

Subject	*Experiment*	*L*	*R*	*L–R*	*(L–R)/L (%)*	*Reduction in sensitivity to arecoline (%)*
1	A	3.46	2.92	0.54	15.6	21.1
	B	3.57	2.26	1.31	36.7	
	C	3.78	3.52	0.26	6.9	
2	A	2.60	2.70	−0.10	−3.8	42.9
	B	2.76	1.68	1.08	39.1	
	C	2.80	2.72	0.08	2.9	
3	A	2.82	2.51	0.31	11.0	6.6
	B	2.96	2.44	0.52	17.6	
	C	3.10	2.90	0.20	6.5	
4	A	2.26	2.18	0.08	3.5	17.1
	B	2.82	2.24	0.58	20.6	
	C	1.58	1.66	−0.08	−5.1	
5	A	3.86	3.86	0	0	18.9
	B	4.34	3.52	0.82	18.9	
	C	3.76	3.88	−0.12	−3.2	
6	A	2.46	2.64	−0.18	−7.3	11.6
	B	2.78	2.66	0.12	4.3	
	C	2.18	2.48	−0.30	−13.8	
7	A	3.04	3.14	−0.10	−3.3	23.5
	B	2.58	2.06	0.52	20.2	
	C	2.66	1.88	0.78	29.3	

minus R difference in miosis was 0.12 ± 0.13 mm, $n = 7$).

Studies on glaucoma patients

One drop of the prescribed pilocarpine concentration gave maximum pupillary effects as in the experiments with pilocarpine on normal volunteers. Thereafter resting pupil size recovered more quickly than reflex amplitude in all patients (Table 3). After 24 h, miosis had recovered by 73% but the reflex by only 22% (mean percentage of maximum increases measured during the week). After 2 days the miosis and reflex had recovered by 87% and 59% respectively.

In the majority of subjects the largest measurement of pupil diameter was made after 4 days following which the pupils became slightly smaller again. By contrast, the size of the reflex increased progressively during the week.

Discussion

It has been shown previously that miosis to directly acting cholinomimetics is accompanied by a proportional reduction in pupillary light reflex amplitude (Smith & Smith, 1978). In the present study it has been found that physostigmine, acting indirectly, also reduced reflex responses though to a lesser extent, presumably because such responses are modified by enhanced activity of the acetylcholine released during the reflex.

Pupillary measurements made after recovery from miosis showed that all the drugs studied had an after-effect on light reflex sensitivity as had been reported earlier with pilocarpine (Newsome & Loewenfeld, 1974). The magnitude of this after-effect was related to the time for which each drug maintained the pupil at its minimum size. Thus aceclidine and pilocarpine had the greatest, and arecoline the least, after-effect. With all four drugs the miotic response was followed by a slight mydriatic overshoot which was most obvious after aceclidine. The after-effect of aceclidine was furthermore accompanied by a decrease in the miotic response to arecoline.

These findings indicate that the aftermath to the action of cholinoceptor miotic agents involves decreased responsiveness of the pupil to further agonist applications, viz. to the small amounts of acetylcholine released in darkness (causing the mydriatic overshoot), to the larger amounts released during the light reflex, and to arecoline applied topically. This decreased responsiveness could be due either to a local change at the sphincter pupillae reducing the effects of cholinoceptor stimulation or to an increase in sympathetic drive to the dilator pupillae. The latter seems unlikely, however, since the after-effect to pilocarpine still occurs in eyes functionally deprived of their sympathetic innervation by prior guanethidine treatment (Newsome & Loewenfeld, 1974). The fact that the subsensitivity was slowly reversible and related to the duration of maximal stimulation suggests that the most likely mechanism was one of receptor desensitization.

Table 3 Resting pupil diameter and light reflex amplitude in 7 glaucoma patients during withdrawal of pilocarpine eyedrop treatment

Patient	*Previous pilocarpine therapy*		*Pupil measurement*	*Peak effects**	*Days following final eyedrops*			
	Conc. (%)	*Duration*	*(mm)*		*1*	*2*	*4*	*7*
1	4	2 weeks	Resting diameter	1.40	2.29	3.56	3.87	3.78
			Reflex amplitude	0	0.09	0.55	0.82	1.04
2	4	2 weeks	Resting diameter	2.01	2.71	3.86	4.67	4.59
			Reflex amplitude	0	0.15	0.44	0.84	1.25
3	2	18 months	Resting diameter	1.29	3.56	4.13	4.46	4.46
			Reflex amplitude	0	0.68	1.18	1.32	1.41
4	4	1 week	Resting diameter	1.10	2.59	3.27	4.00	3.64
			Reflex amplitude	0	0.19	0.69	—	1.04
5	2	9 months	Resting diameter	1.76	5.11	5.30	5.32	4.96
			Reflex amplitude	0	0.57	0.77	—	1.18
6	4	1 week	Resting diameter	2.00	3.14	5.46	7.08	6.38
			Reflex amplitude	0	0.12	0.35	—	1.32
7	2	2 weeks	Resting diameter	2.10	3.90	5.39	5.20	5.39
			Reflex amplitude	0.06	0.18	0.67	—	1.05

* Peak effects measured 40 min after final application of pilocarpine.
In each patient only left pupillary measurements are shown as both pupils responded similarly.
A dashed line indicates that reflex size was not measured.

Patients studied during withdrawal of chronic pilocarpine therapy showed a similar pattern of pupillary subsensitivity to light that reversed slowly over the following week. There was evidence of an overshoot mydriasis since the largest measurement of resting pupil size was made 4 days after stopping pilocarpine, following which the diameter decreased slightly. However, there was no indication that chronic therapy for long periods produced greater degrees of desensitization than that following a single dose of cholinoceptor agonist.

This demonstration of desensitization occurring readily in a human smooth muscle *in situ* could be of clinical importance. It is known that progressive resistance to miotics occurs in glaucoma therapy, and although their ocular hypotensive action depends on ciliary muscle and not iris muscle contraction, there is evidence in monkeys that ciliary muscle subsensitivity occurs (Kaufman & Barany, 1977; Kaufman, 1978) which could originate likewise from receptor desensitization.

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