Consensual pupillary responses to mydriatic and miotic drugs

N. THEOFILOPOULOS, J. LONGMORE, F. A. KERR, E. SZABADI & C. M. BRADSHAW

Department of Psychiatry, University of Manchester, Stopford Building, Oxford Road, Manchester M13 9PT

1 Three experiments were conducted to examine whether mydriatic or miotic drugs instilled into one eye have any effect on the diameter of the pupil of the untreated fellow eye, in healthy volunteers.

2 In *Experiment 1*, the effects of six different concentrations of phenylephrine hydrochloride (0.06–1.50 M) were studied in four subjects, using photography in an illuminated room to assess pupil diameter. The drug evoked a dose-dependent mydriasis in the index eye which was accompanied by a simultaneous dose-dependent miosis in the fellow eye.

3 In *Experiment 2*, the same method was used to assess pupil diameter as in Experiment 1. The effects of mydriatic (methoxamine and tyramine) and of miotic (pilocarpine) drugs instilled into the fellow eye, were studied on the sizes of pupillary responses to the same drugs instilled into the index eye. The presence of a mydriatic drug in the fellow eye resulted in a decrease in the size of the mydriatic responses in the index eye.

4 In *Experiment 3*, the effects of three concentrations of phenylephrine hydrochloride (0.15–0.60 M) and of three concentrations of pilocarpine hydrochloride (0.002–0.008 M), were studied in darkness using an infra-red binocular television pupillometer, in seven subjects. Phenylephrine evoked dose-dependent mydriasis and pilocarpine evoked dose-dependent miosis. The pupillary responses of the index eye were not accompanied by any changes in the diameter of the pupil of the fellow eye.

5 It is concluded that drug-induced mydriasis in the index eye is accompanied by a consensual miosis in the fellow eye. This consensual adjustment in pupil diameter is likely to be due to the operation of the pupillary light reflex, since it is absent when pupil diameter is measured in darkness. Therefore, pharmacologically induced anisocoria cannot be used as a measure of the response to a drug instilled into one eye when pupillary diameter is assessed in an illuminated room.

Keywords mydriasis miosis pupillary light reflex

Introduction

The human pupil provides a convenient *in vivo* test system for the study of the interaction of drugs with either muscarinic cholinceptors or α -adrenoceptors (for review, see Szabadi & Bradshaw, 1986). In such studies, mydriasis or miosis evoked by locally instilled drugs is often assessed as the size of the anisocoria, i.e. the difference between the pupil diameters of the

treated and the untreated eyes. This procedure relies on the assumption that the locally instilled drug would not affect the fellow eye.

However, there have been reports indicating that instillation of a drug into one eye may evoke a change in pupil diameter of the untreated eye. Thus, Bertler & Smith (1971) reported that instillation of phenylephrine into one eye can

Correspondence: Dr E. Szabadi, Department of Psychiatry, University of Manchester, Stopford Building, Oxford Road, Manchester M13 9PT

evoke a miotic response in the fellow eye. On the other hand, Innemee *et al.* (1982) found that clonidine-evoked mydriasis in the treated eye was not accompanied by a change in the pupillary diameter in the fellow eye. Finally, Birmingham *et al.* (1979) reported that pilocarpine-evoked miosis in the treated eye was not accompanied by any detectable change in pupil diameter in the fellow eye.

We report here three experiments addressing the question of consensual changes in pupil diameter in response to local treatment with mydriatic and/or miotic drugs. *Experiment 1* examined the effect of phenylephrine-evoked mydriasis in one eye on pupillary diameter in the fellow eye. *Experiment 2* examined the effects of methoxamine- or tyramine-evoked mydriasis or pilocarpine-evoked miosis in one eye on the sizes of the mydriatic or miotic responses, evoked by the same drugs in the other eye. The reason for conducting this experiment was to examine whether instillation of a mydriatic or a miotic drug into one eye might have an effect, not only on the baseline pupil diameter of the fellow eye, but also on the magnitude of pharmacological responses evoked in that eye. *Experiment 3* was similar in conception to *Experiment 1*, with the exception that it was conducted in darkness in order to assess the extent to which the pupillary light reflex may have contributed to any observed consensual changes in pupil diameter. The experiments were approved by the Ethics Committee of Withington Hospital, Manchester. Some of these results have been communicated to the British Pharmacological Society (Theofilopoulos *et al.*, 1986).

Methods

Subjects

In *Experiment 1*, the subjects were four drug-free healthy volunteers (three females, one male), aged 18–30 years. In *Experiment 2*, the subjects were six drug-free healthy female volunteers, aged 18–23 years. In *Experiment 3*, the subjects were seven drug-free healthy volunteers (three females, four males), aged 19–35 years. All subjects had light-coloured irides and had no previous history of eye disease.

Drugs

In *Experiment 1*, the following freshly made drug solutions were used: 10 µl phenylephrine hydrochloride 0.06 M, 0.125 M, 0.25 M, 0.5 M, 0.75 M and 1.5 M, pH 5.5) were instilled into the conjunctival sac of the right eye, according to the

experimental design (see below). Artificial tear (10 µl, Hypromellose Eyedrops BPC) were instilled into the conjunctival sac of the left eye.

In *Experiment 2*, the following freshly made drug solutions were used: sodium chloride (0.154 M; physiological saline), tyramine hydrochloride (0.072 M, pH 4.0), methoxamine hydrochloride (0.04 M, pH 5.0) and pilocarpine hydrochloride (0.02 M, pH 4.5). One drop, 20 µl, of each of these solutions was instilled into the conjunctival sac according to the experimental design (see below).

In *Experiment 3*, the following freshly made drug solutions were used: phenylephrine hydrochloride (0.15 M, 0.30 M and 0.60 M, pH 5.5) and pilocarpine hydrochloride (0.002 M, 0.004 M and 0.008 M, pH 4.5). 10 µl of each of these solutions were instilled into the conjunctival sac of the left eye according to the experimental design (see below). Artificial tear (10 µl Hypromellose Eyedrops BPC) were instilled into the conjunctival sac of the right eye.

Measurement of pupil diameter

In *Experiments 1 and 2*, pupil diameter was measured using the photographic technique described by Sneddon & Turner (1969).

In *Experiment 3*, pupil diameter was assessed in darkness using an infra-red binocular television pupillometer (Gulf & Western).

Experimental design

In *Experiment 1*, each subject participated in six experimental sessions separated by weekly intervals. In each session the subject was first allowed 30 min to adjust to laboratory lighting conditions. Then, the resting pupil diameter was assessed by taking three photographs of the pupils at 5 min intervals. Then, at time 0 one dose of phenylephrine was administered, from each of the six concentrations corresponding to the six experimental sessions, and photographs were then taken at 5 min intervals over a period of 120 min.

In *Experiment 2*, each subject participated in thirteen experimental sessions. Two sessions took place per week, and at least 48 h were allowed to elapse between successive sessions. In each session, the subjects received one of the following pairs of eye drops (index eye/fellow eye): (1) saline/saline, (2) saline/methoxamine, (3) saline/tyramine, (4) saline/pilocarpine, (5) methoxamine/saline, (6) methoxamine/tyramine, (7) methoxamine/pilocarpine, (8) tyramine/saline, (9) tyramine/methoxamine, (10) tyramine/pilocarpine, (11) pilocarpine/saline, (12) pilocarpine/

methoxamine and (13) pilocarpine/tyramine. The rest of the experimental protocol (laboratory acclimatization, pre- and post-treatment pupil diameter assessments) was similar to the one followed in each session of Experiment 1.

Experiment 3 consisted of two parts. In Part A six subjects participated in three experimental sessions separated by weekly intervals. In each session pupil diameter was assessed in darkness, before and 60 min after the instillation of phenylephrine. Part B was similar to part A, except that pupil diameter was assessed 40 min after the instillation of pilocarpine.

Analysis of data

In *Experiment 1*, the resting pupil diameter was calculated by taking the mean of the three pre-treatment determinations. The size of the mydriatic or miotic response to a dose of phenylephrine was defined as the change in pupil diameter (mm) from the mean of the three pre-drug determinations. For each concentration of phenylephrine instilled, for each subject, and for each pupil, the difference between each post-drug pupillary response and the corresponding mean pre-drug value was first calculated. Then, as visual inspection of the data showed that the mydriatic response to phenylephrine reached a plateau, at all concentrations, approximately 60 min after drug instillation (see below, Results), four maximal responses at 60 min, 65 min, 70 min and 75 min were used to calculate the sizes of both the mydriatic and miotic responses. Thus, for each subject, for each drug concentration and for each pupil, the mean of the four maximal responses was taken, following which the mean ($\pm$ s.e. mean) response of each pupil, to each concentration of phenylephrine and for the whole group of four subjects was calculated.

In *Experiment 2*, a similar procedure was followed, with the exception that the maximal response was obtained by calculating the mean of six maximal determinations per session.

In *Experiment 1*, the dose-response relationships were analyzed by fitting to the data the best-fit rectangular hyperbolic function, using an iterative procedure based on the method of Wilkinson (1961). This method provides estimates ($\pm$ s.e. est.) of the maximal pupillary response ($E_{\max}$) and of the concentration of the instilled drug required to evoke the half maximal response (ED_{50}). The index of determination (p^2) was also calculated; p^2 expresses the proportion of variance in the y-values which can be accounted for in terms of x in a curvilinear function (Lewis, 1960). A log dose-response curve was then plotted using a semilogarithmic

transformation of the best fit rectangular hyperbola.

In *Experiment 2*, all significance levels were calculated using either the paired or unpaired *t*-test, a probability level of $P < 0.05$ (two-tailed) being taken as statistically significant.

Results

Experiment 1

The size of the mydriatic response to the six concentrations of phenylephrine is shown in Figure 1. It can be seen that the size of this response was dose-dependent, and that a maximal response occurred about 60 min after drug instillation. There was a simultaneous consensual miotic response of the left (untreated) eye, and the size of this response to the same six doses of phenylephrine is also shown in Figure 1. It can be seen that the consensual miotic response to phenylephrine was also dose-dependent. The log dose-response curves for the responses to phenylephrine of both pupils are shown in Figure 2, from which it can be seen that both the mydriatic response and the consensual miotic response to phenylephrine were dose-dependent. The parameters of these fitted dose-response curves are shown in Table 1. It can be seen that phenylephrine evoked a maximal dilatation of about 3 mm in the treated eye, while the maximal consensual miosis was 0.67 mm. Both dilatatory and constrictor responses had very similar ED_{50} values. Finally, the values of p^2 indicate that the fitted functions accounted for more than 90% of the data variance.

Experiment 2

The results are shown in Table 2. While physiological saline had no detectable effects on resting pupil diameter, methoxamine and tyramine both dilated the pupil by about 2 mm, and pilocarpine constricted the pupil approximately to the same extent. These responses were affected by the presence of active drugs in the fellow eye. Thus, consistent with the results of the previous experiment, dilatation of the pupil of the fellow eye was accompanied by a consensual miosis of the saline-treated index eye. However, when the fellow eye was treated with pilocarpine there was no significant mydriasis evoked in the saline-treated index eye. In the presence of tyramine in the fellow eye, the mydriatic response of the index eye to methoxamine was significantly attenuated. However, constriction of the pupil of the fellow eye by pilocarpine had no effect on

Figure 1 Time-course of change in pupillary diameter after the instillation of 10 μ l phenylephrine hydrochloride into the conjunctival sac of the right eye, at time 0. Concentration of phenylephrine hydrochloride solution: $\circ$ 0.05 M; $\square$ 0.125 M; $\blacksquare$ 0.25 M; ∇ 0.5 M; $\blacktriangledown$ 0.75 M; $\bullet$ 1.5 M. Pupil diameter was assessed at 5-min intervals with photography in an illuminated room. a) right (treated) eye, b) left (untreated) eye. Ordinate: change in pupil diameter from pre-treatment level (mm), abscissa: running time (min). Each point is the mean of values obtained in four subjects; vertical bars are s.e. mean. Phenylephrine evoked a dose-dependent mydriasis in the right eye that was accompanied by a dose-dependent consensual miosis in the left eye.

the response of the index eye to methoxamine. The response of the index eye to tyramine tended to be attenuated when the fellow eye was treated with methoxamine, but this interaction did not achieve statistical significance. Treatment of the fellow eye with pilocarpine had no significant effect on the response of the index eye to tyramine. Finally, treatment of the fellow eye with mydriatic drugs, had no significant effect on the miotic response of the index eye to pilocarpine.

Experiment 3

The effects of phenylephrine and pilocarpine on

pupil diameter measured in darkness are shown in Figure 3. Phenylephrine and pilocarpine caused significant changes in the diameter of the pupil of the treated eye; however, instillation of these drugs had no significant effect on pupil diameter of the untreated eye.

Discussion

The results of Experiment 1 not only confirm the dose-dependent mydriatic effect of phenylephrine (Haddad *et al.*, 1970), but also show that simultaneously with this mydriatic effect, a miotic

Figure 2 Dose-response curves to phenylephrine hydrochloride instilled into the conjunctival sac of the left eye. Upper curve: mydriatic response in the right eye; lower curve: consensual miotic response in the untreated left eye. Ordinate: change in pupil diameter (mm), abscissa: concentration of phenylephrine hydrochloride solution (M). The volume of solution instilled was 10 μ l for each application. Points are mean values obtained in the group of four subjects, vertical bars are s.e. mean. Curves are semilogarithmic transformations of rectangular hyperbolic functions fitted to the data by an interactive procedure (see text).

response was evoked in the untreated eye, and this response also appeared to be dose-dependent. The results from Experiment 2 also indicate that a similar miotic response in the index eye occurs in the presence of either tyramine hydrochloride or methoxamine hydrochloride in the fellow eye. These findings are in keeping with the report of Bertler & Smith (1971). The most likely explanation for this consensual miotic response is that it is due to the consensual light reflex, which has been shown to be still active during the mydriatic response to sympathomimetic amines (Loewenfeld, 1958). Consequently, following instillation of a mydriatic drug into one eye, more light will reach the retina of this eye, and afferent signals will be sent to both third nerve nuclei. This will result in a parasympathetically-mediated pupil constriction of both eyes. This miotic effect will be seen more readily in the untreated eye than in the treated eye the pupil of which has been dilated. Furthermore, Experiment 2 shows that a mydriatic drug instilled into one eye attenuates the response to another mydriatic drug instilled into the other eye. Therefore experiments in which pharmacological responses are evoked simultaneously in the two eyes may be prone to producing misleading results.

Interestingly, a miotic response evoked by pilocarpine in one eye was not accompanied by a

Table 1 Parameters of the dose-response curves in Figure 2; $\pm$ s.e. est.

	E_{max} Change in pupil diameter (mm)	ED_{50} Concentration of phenylephrine (M)	p^2
Mydriatic response to phenylephrine	$+3.77 \pm 0.18$	0.14 ± 0.02	0.97
Miotic (consensual) response	-0.68 ± 0.06	0.18 ± 0.05	0.93

Table 2 Pupillary responses of 'index' eye, mm (mean $\pm$ s.e. mean; $n = 6$)

Drug instilled into 'index eye'	Drug instilled into 'fellow eye'			
	Saline	Methoxamine	Tyramine	Pilocarpine
Saline	0.02 ± 0.30	$-0.36 \pm 0.11^*$	$-0.30 \pm 0.07^{**}$	0.15 ± 0.92
Methoxamine	2.09 ± 0.28	—	$1.65 \pm 0.25^{***}$	2.09 ± 0.28
Tyramine	2.23 ± 0.30	2.15 ± 0.26	—	2.47 ± 0.24
Pilocarpine	-1.97 ± 0.38	-2.33 ± 0.34	-2.40 ± 0.31	—

* $P < 0.05$, ** $P < 0.02$, *** $P < 0.01$ (Student's t -test)

Figure 3 Change in pupil diameter 60 min after the instillation of 10 μ l phenylephrine hydrochloride (top graph) and 40 min after the instillation of 10 μ l pilocarpine hydrochloride (bottom graph) into the conjunctival sac of the left eye. Concentrations of drug solutions as indicated. Open columns: left (treated) eye, closed columns: right (untreated) eye. Heights of columns indicate mean values ($n = 6$); vertical bars are s.e. mean. Asterisks denote statistically significant changes ($P < 0.05$). Phenylephrine-evoked mydriasis or pilocarpine-evoked miosis was not accompanied by any change in the diameter of the pupil of the untreated fellow eye.

consensual mydriasis in the other eye. The decrease in the illumination of the retina following the pilocarpine-evoked miosis may not constitute a sufficiently strong stimulus to elicit a reflex adjustment of the size of the pupil of the fellow eye. Further experiments, for example using different levels of ambient illuminations, might help to substantiate this suggestion.

Finally, the results of Experiment 3 indicate that the consensual pupillary response is abolished in darkness. Consequently, one observes the effects of the instilled drugs only in the treated eye.

In conclusion, the present experiments demonstrate a consensual adjustment in pupil

size after mydriasis (or miosis) in one eye. This consensual adjustment would tend to exaggerate the pharmacologically induced anisocoria. Thus, anisocoria is not a reliable measure of the size of the pupillary dilatation or constriction evoked by locally instilled drugs in an illuminated room. To avoid the confounding role of the consensual adjustment, measurements should either be carried out in darkness, using infra-red television pupillometry, or the change in pupil size should be assessed as the difference between pre-treatment and post-treatment pupil diameters.

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