The Human Pupil as a Model for Clinical Pharmacological Investigations

PAUL TURNER, MD, FRCP, Professor of Clinical Pharmacology, St Bartholomew's Hospital, London

Experimental systems for the study of autonomically-acting drugs in man are limited by criteria of safety and patient acceptability. Although many invasive techniques are safe when carried out under the supervision of experienced investigators, they may not be sufficiently acceptable to the patient to encourage him to tolerate them on repeated occasions. This has encouraged clinical pharmacologists to develop non-invasive and acceptable techniques that are sensitive enough to demonstrate meaningful pharmacological activity of established and new drugs.

The human eye, and in particular the pupil, has been used for many years to demonstrate the actions and interactions of cholinergic and anti-cholinergic drugs. The pupil is ideal for such purposes, being supplied by both sympathetic and parasympathetic divisions of the autonomic system. It is also readily measurable by a variety of both simple and sophisticated techniques. Furthermore, its responses to cholinergic drugs and to sympathomimetic amines are consistent within subjects when repeated under standard conditions.

The possibility of using the human pupil to study adrenergic neurone and receptor pharmacology arose when it was found that the local administration of guanethidine to the eye was of value in at least two ophthalmic conditions, namely glaucoma (Oosterhuis, 1962) and hyperthyroid lid retraction (Sneddon and Turner, 1966; Crombie and Lawson, 1967; Gay and Wolkstein, 1966). Guanethidine not only reduced the width of the palpebral fissure but also produced a miosis and changes in pupillary responsiveness to sympathomimetic amines.

EXPERIMENTAL METHODS

Several methods are available for measuring pupil responses. The best, but most expensive, is infra-red pupillography (Lowenstein and Loewenfeld, 1958), which has recently been elaborated further by using a closed circuit television system to observe the eye and a signal processor to measure and display pupil diameter. A low-intensity near infra-red source illuminates the

eye without discomfort or distraction to the subject (Whittaker Corporation, Space Sciences Division). This method is required for measurement and recording of rapid changes in pupillary diameter, and when pupil reactivity to light and dark are being observed. Where slower responses are being studied, however, this is not usually necessary, and simpler photographic methods are equally acceptable (Sneddon and Turner, 1967), in which pupil diameters are measured from photographic negatives projected on to a white screen or film viewer. In fact, measurement of pupil size under standard conditions by comparison with a pupil chart (0·5 mm steps) may be sufficiently accurate to provide meaningful information (Allum *et al.*, 1974).

For most purposes it is sufficient to instil a drug in a known concentration into the conjunctival sac as one or more drops from a glass or plastic dropper under standard conditions. It is of interest, however, that Smith (1974) has demonstrated that satisfactory dose-response relationships could be observed with tropicamide concentrations of 1·25 to 40 μg/ml when the eye was exposed to the drug solution over 100 minutes, by comparison with the concentrations of 5 to 10 mg/ml, which are usually applied as eye drops.

ADRENERGIC NEURONE BLOCKING DRUGS

Local Administration

Guanethidine is an adrenergic neurone blocking drug, inhibiting the release of noradrenaline by sympathetic nerve stimulation. It was found that sympathomimetic amines could be divided into two groups on the basis of their interactions with guanethidine instilled locally into the eye. The mydriasis produced by the indirectly-acting amines ephedrine, hydroxyamphetamine, racemic amphetamine and phenmetrazine in the control eye was abolished by pre-treatment with guanethidine 10 per cent in the other eye. In contrast, the mydriatic effects of the directly-acting amines methoxamine, adrenaline and phenylephrine were markedly potentiated in the treated eye (Sneddon and Turner, 1967). The close similarity of these results in man with guanethidine to those reported following chronic sympathectomy of the cat iris (Marley, 1962) suggests that a common mechanism, such as noradrenaline depletion, may be responsible for the altered responses to sympathomimetic amines following chronic treatment with high concentrations of guanethidine. In keeping with this were observations in three patients who developed Horner's syndrome after unilateral cervical sympathectomy in whom there was marked reduction in mydriasis produced by hydroxyamphetamine and ephedrine, and potentiation of that to phenylephrine (Turner, 1969).

Debrisoquine resembles bretylium in producing adrenergic neurone blockade without catecholamine depletion (Abrams *et al.*, 1964) and its use in man

is associated with exaggerated pressor responses to tyramine and noradrenaline. Studies in patients in whom debrisoquine 2 per cent drops were instilled daily for 28 days showed that there was potentiation of phenylephrine mydriasis as with guanethidine, but whereas ephedrine mydriasis disappeared in the guanethidine-treated eye, it persisted in the eye treated with debrisoquine (Sneddon and Turner, 1968), indicating the continued presence of catecholamines.

Bethanidine demonstrates a biphasic action, a low concentration producing a significant potentiation of phenylephrine mydriasis without a change in ephedrine response. High concentrations produced potentiation of phenylephrine mydriasis and suppression of that with ephedrine (Lind and Turner, 1969). These findings are consistent with those of Costa *et al.* (1962) in the cat.

Oral Administration

Studies in hypertensive patients successfully treated with oral reserpine, methyldopa, guanethidine and bethanidine demonstrated a significant decrease in mean pupil diameter compared with pre-treatment readings (Sneddon, 1968). Serial determinations of ephedrine mydriasis in such patients before and after treatment with these antihypertensive drugs showed that reserpine and methyldopa significantly reduced ephedrine mydriasis, but guanethidine and bethanidine tended to increase it (Sneddon, 1968). The reduction by reserpine and methyldopa may be explained by noradrenaline depletion from the sympathetic nerve endings in the pupil. The effects of guanethidine and bethanidine suggest that in antihypertensive doses they produce their effects without producing noradrenaline depletion.

ADRENERGIC RECEPTOR BLOCKING DRUGS

Local Administration

Pupil dilatation is an α-adrenergic receptor-mediated effect. Few α-receptor blocking drugs are suitable for ocular administration. Dibenamine and phentolamine have been administered by this route but are irritating and poorly tolerated. Thymoxamine is a selective α-adrenergic blocking drug without effects on β-receptors (Foster, 1966) which is tolerated in the eye in concentrations up to 0·5 per cent. Instillation in the eye produces a miosis which is present for up to 24 hours, and is accompanied by a reduction in the width of the palpebral fissure for the first few hours. Pre-treatment of one eye with thymoxamine prevents the mydriatic action of phenylephrine and ephedrine seen in the untreated eye. When mydriasis has been established with hydroxyamphetamine, thymoxamine reverses it (Turner and Sneddon, 1968).

Oral Administration

Oral administration of thymoxamine 50 mg produced a significant reduction in phenylephrine-induced mydriasis (Arbab and Turner, 1970).

Indoramin is an antihypertensive drug with competitive α-adrenoceptor blocking properties (Royds *et al.*, 1972; White *et al.*, 1974). Oral administration of 20 and 40 mg produced a reduction of phenylephrine-induced mydriasis, followed by a dose-related miosis (Coltart *et al.*, 1971).

MONOAMINE OXIDASE INHIBITORS

Important among the interactions of monoamine oxidase inhibitors are those with indirectly acting sympathomimetic amines such as tyramine and hydroxyamphetamine. Studies in depressed patients have shown that treatment with these drugs produces a marked increase in tyramine and hydroxyamphetamine mydriasis. The usefulness of this experimental model was confirmed by the demonstration that clorgyline, a new monoamine oxidase inhibitor, not only increased tyramine and hydroxyamphetamine mydriasis but also potentiated the systemic pressor response to oral phenylpropanolamine (Bevan-Jones and Lind, 1971).

OTHER INTERACTIONS

Among other interactions that have been studied on the human pupil must be mentioned antagonism of morphine-induced miosis by nalorphine when both are applied locally to the eye (Normof *et al.*, 1968).

STUDIES WITH METABOLITES

Pupillary changes after administration of a drug by mouth may be due to the action of a metabolite rather than the parent compound. An example of this is the mydriasis that has been described after fenfluramine administration, particularly in large doses (White *et al.*, 1967; Griffith *et al.*, 1974). Local administration of fenfluramine 1 per cent as eye drops did not produce pupillary dilatation in human volunteers (Turner, 1970). Norfenfluramine, the principal metabolite of fenfluramine, however, produced a dose-related mydriasis when instilled in the eye of volunteers, which was reduced or abolished by local treatment with thymoxamine (Kramer *et al.*, 1973). This appeared to be a direct sympathomimetic action of norfenfluramine, as pre-treatment of one eye with guanethidine eye drops for five days was followed by a norfenfluramine-induced mydriasis identical to that in the untreated control eye. Furthermore, oral administration of fenfluramine to hypertensive patients treated with reserpine produced a marked mydriasis (Fig. 1), the time course of which is more closely related to plasma levels of

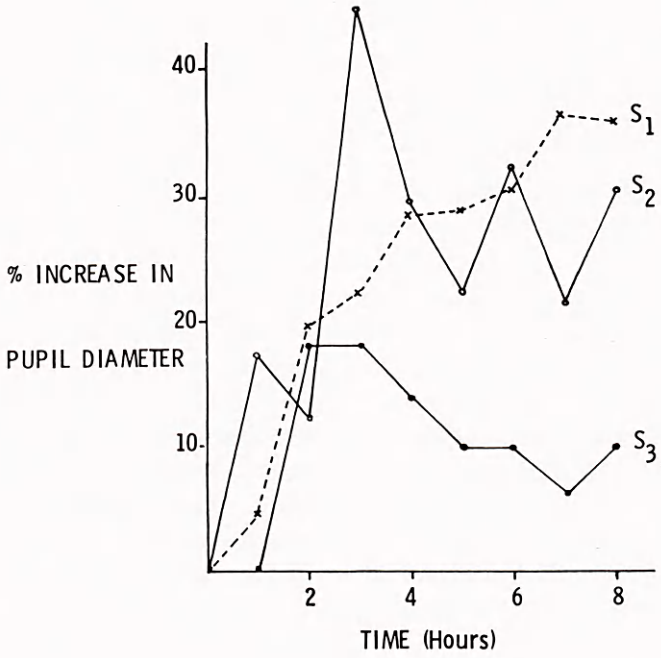

Fig. 1. Percentage increase in pupil diameter in three patients receiving reserpine for hypertension following the oral administration of fenfluramine 40 mg.

norfenfluramine than fenfluramine (Campbell, 1971). It is probable, therefore, that fenfluramine-induced mydriasis is produced by the metabolite norfenfluramine, which is known from animal studies to possess pharmacological properties different from the parent compound (Jori *et al.*, 1973; Costa and Revuelta, 1972).

NEURONE MATURATION

There is considerable controversy regarding the state of maturity of various parts of the autonomic nervous system in newborn infants. Studies of pupillary responses have provided some idea of the maturity of the innervation of the iris at different stages of gestational age. A mydriatic response was always obtained with the directly-acting sympathomimetic amine phenylephrine from 28 weeks gestational age onwards, and there was no correlation between degree of mydriasis and gestational age. When hydroxyamphetamine or tyramine, indirectly acting amines, were used, the most premature babies tended not to respond, and there was a significant correlation between the magnitude of mydriasis and gestational age. These results indicated that the

end-organ, the iris, had the capacity to respond to the neurotransmitter, but that the synthesis, storage or release of neuronal noradrenaline was deficient (Lind *et al.*, 1971). The anticholinergic drug eucatropine produced a mydriasis from the earliest gestational age studied, the magnitude of mydriasis being unrelated to age, suggesting cholinergic neurone and receptor maturity from that early date. These results have more recently been confirmed by Carpel and Kalina (1973).

THYROID STATUS AND ADRENERGIC ACTIVITY

There is considerable evidence that many of the peripheral manifestations of hyperthyroidism are mediated through sympathetic activity (Turner, 1974). Serial determinations of ephedrine mydriasis were carried out in hyperthyroid and hypothyroid patients before and after treatment. During the period of treatment with antithyroid drugs there was a significant reduction in ephedrine mydriasis in the hyperthyroid patients, and a significant increase in hypothyroid patients during treatment with thyroxine (Turner, 1969, 1972). These results are further evidence for an interaction between thyroid hormones and adrenergic activity.

NEUROPATHIES

Pupil responses to sympathomimetic amines and parasympathomimetic drugs have been used for many years in the diagnosis and investigation of Horner's syndrome and Holmes-Adie syndrome. Hopkins *et al.* (1974) have described two patients with evidence of acute autonomic failure including postural fainting, paralysis of accommodation, and failure of sweating and lachrymation. They both showed a brisk constrictor response to methacholine 2·5 per cent, suggesting denervation on the cholinergic side, and a slight mydriasis in one patient to adrenaline 1 per cent, suggesting a partial adrenergic denervation.

A more generalised neurological condition is familial dysautonomia (Riley-Day syndrome) characterised by vasomotor instability, poor muscle co-ordination, and impairment of sweating, lachrymation and temperature control. There are exaggerated vascular responses to injections of methacholine and noradrenaline. We have studied the pupil responses of a child of eighteen months with this condition, and compared them with those of control children matched for age and eye colour. Methacholine 2·5 per cent and physostigmine 0·5 per cent eye drops produced a significantly greater miosis in the patient compared with the control subjects. The mydriasis produced by phenylephrine was also greater in the patient, but ephedrine, which produced a mydriasis in the controls, did not have a measurable effect in the

patient (Shinebourne *et al.*, 1967). These responses are characteristic of denervation of the sympathetic and parasympathetic systems, and are consistent with observations in the cardiovascular and other systems.

CONCLUSIONS

Measurement of pupillary responses to autonomic drugs may provide a simple and satisfactory method for studying their action and interaction in man. Pupil responses may also be used for testing the integrity of the autonomic supply to the eye, both from the point of view of its maturation, and also of its involvement in pathological processes.

Acknowledgements

I am indebted to my colleagues who have collaborated with me over several years, and to the Mental Health Research Fund who provided a grant towards equipment used in these studies.

This article is based on a paper read at a Growing Points in Medicine meeting held in the Royal College of Physicians in April 1974.

References

Abrams, W. B., Pocelinko, R., Klausner, M., Hanauer, L. and Whitman, E. N. (1964) *Journal of New Drugs*, **4**, 268.
Allum, W., Aminu, J., Bloomfield, T. H., Davies, C., Scales, A. H. and Vere, D. W. (1974) *British Journal of Clinical Pharmacology*, **1**, 51.
Arbab, A. G. and Turner, P. (1970) *Journal of Pharmacy and Pharmacology*, **22**, 532.
Bevan-Jones, B. and Lind, N. A. (1971) *British Journal of Pharmacology*, **41**, 428P.
Campbell, D. B. (1971) *British Journal of Pharmacology*, **43**, 465P.
Coltart, D. J., Lockhart, J. D. F., Royds, R. B. and Turner, P. (1971) *British Journal of Pharmacology*, **43**, 467P.
Carpel, E. F. and Kalina, R. E. (1973) *American Journal of Ophthalmology*, **75**, 988.
Costa, E. and Rovuelta, A. (1972) *Biochemical Pharmacology*, **21**, 2385.
Costa, E., Kuntzman, R., Gessa, F. L. and Brodie, B. B. (1962) *Life Science*, **3**, 75.
Crombie, A. L. and Lawson, A. H. H. (1967) *British Medical Journal*, **4**, 592.
Foster, R. W. (1966) *Journal of Pharmacy and Pharmacology*, **18**, 1.
Gay, A. J. and Wolkstein, M. A. (1966) *Archives of Ophthalmology*, **76**, 364.
Griffith, J. D., Wittl, J. G. and Jasinski, D. R. (1974) *Clinical Pharmacology and Therapeutics*, **15**, 207.
Hopkins, A., Neville, B. and Bannister, R. (1974) *Lancet*, **1**, 769.
Jori, A., Dolfini, E., Tognoni, G. and Garattini, S. (1973) *Journal of Pharmacy and Pharmacology*, **25**, 315.
Kramer, R., Rubicek, M. and Turner, P. (1973) *Journal of Pharmacy and Pharmacology*, **25**, 575.
Lind, N. A. and Turner, P. (1969) *British Journal of Pharmacology*, **35**, 376P.
Lind, N. A., Shinebourne, E. and Turner, P. (1971) *Pediatrics*, **47**, 105.
Lowenstein, O. and Loewenfeld, I. E. (1958) *Archives of Ophthalmology*, **59**, 352.
Marley, E. (1962) *Journal of Physiology*, **162**, 193.
Normof, N., Elliot, H. W. and Parker, K. D. (1968) *Clinical Pharmacology and Therapeutics*, **9**, 358.
Oosterhuis, J. A. (1962) *Archives of Ophthalmology*, **67**, 802.
Royds, R. B., Coltart, D. J. and Lockhart, J. D. F. (1972) *Clinical Pharmacology and Therapeutics*, **13**, 380.
Shinebourne, E., Sneddon, J. M. and Turner, P. (1967) *British Medical Journal*, **4**, 91.
Smith, S. E. (1974) *British Journal of Clinical Pharmacology*, **1**, 37.
Sneddon, J. M. (1968) Ph.D thesis. University of London.
Sneddon, J. M. and Turner, P. (1966) *Lancet*, **2**, 525.
Sneddon, J. M. and Turner, P. (1967) *Journal of Physiology*, **189**, 20P.
Sneddon, J. M. and Turner, P. (1968) *British Journal of Pharmacology*, **32**, 432P.

Turner, P. (1969) *Triangle*, **9**, 91.
Turner, P. (1970) In *International Symposium on Amphetamines and Related compounds*. (Ed. E. Costa and S. Garattini). New York: Raven Press.
Turner, P. (1972) *Proceedings of fifth International Congress on Pharmacology*, San Francisco.
Turner, P. (1974) *Drugs*, **7**, 48.
Turner, P. and Sneddon, J. M. (1968) *Clinical Pharmacology and Therapeutics*, **9**, 45.
White, A. G., Beckett, A. H. and Brookes, L. G. (1967) *British Medical Journal*, **1**, 740.
White, C., Royds, R. B. and Turner, P. (1974) *Postgraduate Medical Journal* (in press).

Book Review

Malabsorption in Clinical Practice by M. S. Losowsky, B. E. Walker and J. Kelleher. Churchill Livingstone (1974). Price £8. 318 pages.

The most valuable features of this expensive little book are the list of references and the co-authorship of a qualified biochemist.

There are over 1850 references, some as recent as 1972, and for the convenience of the reader many are grouped into sections within the text; for instance, chapter seven, which deals with the causes of steatorrhoea, contains 43 subsections each followed by a list of relevant references.

The biochemical sections are particularly rewarding for the clinician since, quite apart from the useful introductory chapters, the laboratory viewpoint is clearly expressed and the pros and cons of various investigations and the techniques employed are carefully weighed; too carefully in many cases where the authors offer no opinion of their own to tip the scales either way. Some cautious suggestions are made; for instance, in the matter of faecal fat the reader gains a distinct impression in favour of gravimetric rather than titrimetric assay and, moreover, that with the intelligent use of continuous markers (but which marker?) there may be as much to be learned from a single stool as from a seven-day balance study. Why then devote a chapter, albeit a short one, to the organisation of a metabolic ward whose main purpose is to facilitate the performance of accurate fat balances? In justice it must be admitted that other balances may be conducted in such a ward, witness the rather droll inference (p. 304) that patients have a habit of micturating in the bath.

There are helpful chapters on bacteriology and on immunity. The purely clinical parts of the book are adequate and even contain some personal experiences of the authors although the stamp of authority is absent. A trifling criticism is that there is virtually no guidance on the details of a gluten-free diet, a matter of little consequence to a clinician working with an experienced dietitian but inconvenient in a situation not involving such a liaison.

J.T.W.