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EFFECTS OF A POTENT AND SPECIFIC β_2-ADRENOCEPTOR ANTAGONIST ON INTRAOCULAR PRESSURE

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1 Physiological studies support the possibility of adrenergic regulation of aqueous humor secretion from the ciliary process, a tissue which has recently been shown to contain a predominance of β_2-adrenoceptors.

2 The present experiments examine the effects of a potent and highly specific β_2-antagonist (IPS 339) on intraocular pressure in the normal rabbit eye.

3 The observed decrease in pressure caused by IPS 339 suggests that highly specific β_2-antagonists may be useful in decreasing elevated intraocular pressure.

Introduction

Nonspecific β-adrenoceptor antagonists, such as timolol, are able to decrease intraocular pressure (IOP) both in animals and man (Zimmerman & Boger, 1979). Although these agents have been found useful in the treatment of open angle glaucoma, their mechanism of action is incompletely understood. Many physiological studies suggest that β-antagonists decrease aqueous humor formation through an effect on the ciliary processes, which are located in the posterior ocular chamber (Neufeld, 1979). Biochemical studies (Waitzman & Woods, 1971; Neufeld & Page, 1977; Bromberg, Gregory & Sears, 1980; Nathanson, 1980a) have shown that there is a substantial enrichment of β-adrenoceptors in the ciliary processes, particularly in the epithelial cells (Nathanson, 1980b), suggesting that β-antagonists may directly affect secretory mechanisms. Such studies indicate, also, that β-receptors present in the ciliary processes have pharmacological characteristics indicative of a predominance of β_2-adrenoceptors (Nathanson, 1980b). This latter finding is of some clinical interest since, if factors such as drug absorption and catabolism are taken into account, it would raise the possibility that potent β_2-adrenoceptor antagonists should be as effective as nonspecific β-blockers in decreasing IOP and also have fewer potential side effects on tissues containing β_1-receptors, such as heart.

Although previous experiments have shown that the β_2-antagonist, butoxamine, is relatively ineffective in decreasing IOP (Rowland & Potter, 1980, see Discussion, below), radioligand binding studies indicate that many existing β_2-antagonists (such as butoxamine) are much less potent in blocking the β-adrenoceptor than non-specific β-antagonists such as propranolol or timolol (Minneman, Hedberg & Molinoff, 1979a; Minneman, Hegstrand & Molinoff, 1979b). Furthermore, these biochemical studies indicate that some β_2-antagonists (such as butoxamine and H35/25), which were previously considered to be fairly specific, show, in fact, only a low degree of selectivity for β_2 versus β_1-receptors (Minneman *et al.*, 1979b).

Recently, Imbs, Miesch, Schwartz, Velly, LeClerc, Mann & Wermuth (1977) have described a potent and highly specific β_2-antagonist, IPS 339 ((*t*-butyl-amino-3-ol-2-propyl) oximino-9-fluorene hydrochloride). Both physiological and biochemical studies (Minneman *et al.*, 1979a) indicate that this agent is about 20 to 100 fold more effective in blocking β_2-adrenoceptors in lung than β_1-receptors in heart. Furthermore, IPS 339 has recently been found to be a potent antagonist of rabbit and human ciliary process β-adrenoceptor-stimulated adenylate cyclase (Nathanson, 1980b; 1981), with a k_i (3×10^{-9}M) comparable to that for the nonspecific β-antagonist, timolol ($k_i = 2.5 \times 10^{-9}$M). The present studies were carried out to determine whether IPS 339 has any physiological effects on decreasing intraocular pressure in the normal rabbit eye.
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Methods

Male (2 to 4 kg), New Zealand white rabbits were housed under standard conditions and exposed to a 12 h light-dark cycle. Intraocular pressure was measured with a Perkins applanation tonometer after topical anaesthesia with 0.4% benoxinate (and 0.25% sodium fluorescein). The tonometer had been calibrated previously by connecting the anterior

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chamber of ennucleated rabbit eyes to a manometer (and reservoir) and taking tonometer readings at different pressures. A large number of preliminary IOP readings were made in order to accommodate the animals to the measurement procedure. These readings indicated reproducible control pressures of 14.02 ± 0.06 mmHg (mean ± s.e. mean for 120 measurements), comparable to those obtained by others (e.g. Bhattacherjee, 1971). To minimize possible diurnal pressure variations, measurements were made at the same time of day for both drug and control eyes. All drug studies were carried out in a blind fashion by two experimenters, one applying the drug (or placebo) and the second measuring IOP. This second experimenter had no knowledge as to which drugs (or placebo) were applied until the end of the entire study.

Four rabbits received (in two, 50 µl doses) either 0.5% IPS 339 (mixed in phosphate buffered saline, pH 7.4) or 0.5% timolol in the inferior conjunctival sac of the left or right eye. The contralateral eye received phosphate buffered saline alone. (Because 0.5% is a frequently employed dose in experimental studies of timolol action, a similar dose was chosen for IPS 339. Preliminary experiments indicated that this dose of IPS 339 could lower IOP without overt ocular toxicity.) IOP measurements were made on both ipsilateral and contralateral eyes at 0, 1, 2, 3, 4, and 6 h after application, by which time pressure in most drug-treated eyes had returned to normal. Two days later the same four rabbits again received IPS 339 or timolol but to the eyes previously treated with phosphate buffered saline, and IOP was measured as above. This procedure was repeated again at 4 and at 6 days, such that by the end of the experiment each eye had received one dose of each of the two drugs and no eye had received the same drug more than once. All 8 eyes, except one, showed consistent responses to drug application. The single eye which did not, failed to respond to either IPS 339 or to timolol. Comparisons between groups and comparisons pre- and post-drug application were calculated both on the basis of including this eye ($n = 8$) or excluding it ($n = 7$). The statistical results described below were significant using either group size.

In addition to these rabbits, four others received no drugs but only phosphate buffered saline for the duration of the experiment. IOP measurements for these rabbits, recorded on the same schedule as those described above, revealed no significant change in baseline IOP during the course of the study.

Results

Figure 1 shows the mean IOP readings for the 6 h following application of either IPS 339 or timolol. Compared to pre-drug readings, both agents caused a

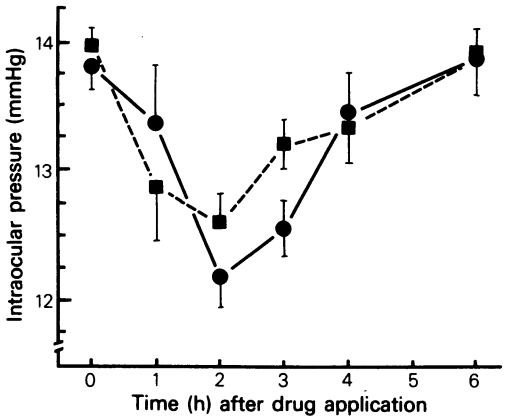

Figure 1 Effect of topically applied 0.5% IPS 339 (●) or 0.5% timolol (■) on intraocular pressure in rabbit eyes. Groups are as described in text. Shown is the mean pressure in mmHg before treatment (0 h) and for 6h following drug application in the ipsilateral eye; vertical lines show s.e. mean.

significant decrease in IOP in ipsilateral eyes at 2 and 3 h after application (d.f. = 7, $P < 0.01$, paired t test); timolol also caused a significant decrease at 1 h compared to pre-drug readings (d.f. = 7, $P < 0.05$). The extent of the decrease caused by the two drugs did not differ significantly except at 3 h, at which time the decrease caused by IPS 339 was significantly more than that caused by timolol (d.f. = 14, $P < 0.05$, unpaired t test). During the course of the experiments no significant changes in pupillary diameter nor overt ocular toxicity were noted for either drug.

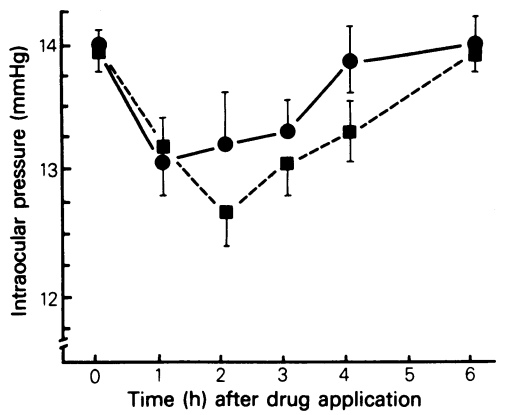

Figure 2 Effect of topically applied 0.5% IPS 339 (●) or 0.5% timolol (■) on intraocular pressure in saline-treated *contra*lateral eyes; vertical lines show s.e. mean. Data shown are for eyes contralateral to the drug-treated eyes shown in Figure 1. The observed decrease in pressure, which was greater for timolol, may be due to systemic absorption of drug, see text.

In addition to decreasing IOP in the ipsilateral eye, IPS 339 also caused a smaller decrease in pressure in the contralateral eye (Figure 2). Compared to baseline readings, this decrease was significant at 1 h (d.f. = 7, $P < 0.05$) but not at 2 or 3 h. Comparing the ipsilateral to the contralateral eye at similar time periods, the magnitude of the decrease caused by IPS 339 was significantly greater in the ipsilateral eye at 2 and 3 h (d.f. = 7, $P < 0.05$). Compared with IPS 339, timolol caused a greater decrease in IOP in the contralateral eye. This decrease for timolol was significantly different from control at 1, 2, 3 and 4 h (d.f. = 7, $P < 0.05$) and was quite similar in degree and duration to the decrease which occurred in the timolol-treated ipsilateral eye. This effect, which has been described previously for timolol (Zimmerman & Boger, 1979), is presumably due to systemic absorption of the drug.

Discussion

These data indicate that the specific β_2-antagonist, IPS 339, can decrease IOP in the normal rabbit eye. The results are consistent with recent biochemical data indicating that IPS 339 is a potent antagonist of β-adrenoceptors in the rabbit and human ciliary processes (Nathanson, 1980b; 1981). At the particular dose employed in the present studies, the magnitude of the decrease in IOP due to IPS 339 was similar to that caused by the nonspecific β-antagonist timolol. Although, at 3 h after application, IPS 339 was somewhat more effective than timolol, no conclusion regarding the relative *in vivo* potencies of these drugs can be drawn until more extensive dose-response curves are obtained.

It has been known for many years that, like β-adrenoceptor antagonists, β-adrenoceptor agonists also decrease intraocular pressure. This overtly paradoxical situation has been attributed to different sites of action of adrenoceptor agonists and antagonists, the former affecting reabsorptive properties and the latter, secretion. (Other views also exist.) Paterson & Paterson (1972) and Langham & Diggs (1974) have found that, among adrenoceptor agonists, the relatively selective β_2 agent, salbutamol, is as effective as isoprenaline in lowering IOP in the normal rabbit eye. Potter & Rowland (1978) have shown, also, that β_2-selective agents such as metaproterenol and salbutamol are more effective than β_1-agonists in decreasing IOP in both the normal and water-loaded rabbit eye (Rowland & Potter, 1980).

Previous studies using selective β-adrenoceptor antagonists are less clear. Thus, although β_1-selective blocking agents can decrease IOP, most studies have shown these agents to be equal or somewhat less potent than propranolol, and much less potent than the non-selective antagonist, timolol (see Zimmerman & Boger, 1979 for summary). In the case of β_2-antagonists, butoxamine has been reported to be ineffective in lowering IOP in the eye of the water-loaded rabbit (Rowland & Potter, 1980). In contrast, H35/25, another β_2-adrenoceptor antagonist, does lower IOP in the normal cat eye (Colasanti & Trotter, 1979). Although species differences may exist, it is interesting, in this regard, that H35/25 ($K_i = 4.3 \times 10^{-7}$M) is about 10 times more potent than butoxamine ($K_i = 4.4 \times 10^{-6}$M) in blocking isoprenaline-stimulated adenylate cyclase in the ciliary process (Nathanson, 1980b).

Both butoxamine and H35/25 have been shown, by biochemical methods, to be relatively poor in discriminating between β_2 and β_1 receptors. For example, the potency ratio (K_i heart/ K_i ciliary process) of these compounds in blocking β-adrenoceptor-stimulated adenylate cyclase in ciliary process (predominately β_2) versus heart (predominately β_1) is 1.5 for butoxamine and 3 for H35/25 (Nathanson, 1980b; Minneman *et al.*, 1979b). This compares with a ratio of greater than 50 for IPS 339. In addition, on an absolute basis, IPS 339 ($K_i = 3 \times 10^{-9}$M) is much more potent than either butoxamine or H35/25.

It will be important in future studies to determine whether IPS 339 can also decrease IOP in conditions of elevated pressure. Preliminary studies in our laboratory with α-chymotrypsin-induced glaucoma in rabbits indicate that this may be the case. However, it is premature to suggest that IPS 339 might be effective in treating glaucoma clinically. The long-term toxicity of this compound is unknown, and acute systemic administration has been reported to cause mild hypertension (Imbs *et al.*, 1977). Rather than necessarily indicating the use of a particular compound, the present results, together with earlier biochemical data, support the concept that potent β_2-adrenoceptor antagonists, as a group, should be investigated further for their possible effectiveness in reducing elevated IOP.

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