Effects of prostaglandins and norepinephrine on ocular pressure and pupil size in rabbits following bilateral cervical ganglionectomy

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We wish to report an early (10 to 30 min) 6 to 7 mm Hg increase in IOP above predrug levels following topical norepinephrine (NE) (5 μmol) administration in conscious normal rabbits. This early elevation in IOP was significantly more pronounced in bilateral superior cervical ganglionectomized (BG) rabbits. The time course for peak IOP elevation slightly preceded the accompanying peak mydriatic effect in all groups. IOP returned to baseline levels in about 60 min in the BG rabbits, but the mydrias persisted. Pretreatment with topical indomethacin significantly reversed the early hypertonic NE effect on IOP in the BG animals implying the involvement of prostaglandin synthesis. The IOP continued to fall significantly after 60 min in the BG group with indomethacin pretreatment. This latter result suggests a predominant long-lasting NE effect. The indomethacin pretreatment had no effect on pupil size of any group, but phenoxybenzamine (PBA) reduced the mydriatic effect, implying a direct or a predominant role of NE on iris muscle.

Keywords: norepinephrine, prostaglandins, intraocular pressure, pupil diameter, cervical ganglionectomy, indomethacin, phenoxybenzamine, catecholamine supersensitivity

Norepinephrine (NE) is known to cause a 6 to 7 mm Hg decrease in intraocular pressure (IOP) 3 to 6 hr following an initial topical application to rabbit cornea and conjunctiva. The time course of this decrease in IOP always succeeded increases in pupil diameter (PD).

On the other hand, changes in the IOP for rabbits during the first 90 min after topical application of NE have not been examined in detail. No studies have reported a significant effect of NE on IOP earlier than 60 min, even after unilateral superior sympathectomy, and no authors have commented upon such an effect. However, a significant increase in IOP has been observed 30 min after 0.1% topical epinephrine. At 60 min after a single NE application, neither decreases nor increases in IOP have been shown to be significant.

The known biphasic responses to epinephrine or multiple topical application of...
NE, as well as time-course studies of topical prostaglandin (PG) effects, suggested to us that the response to single topical applications of NE should be more closely examined during the first 60 min. Previous reports indicate catecholamine supersensitivity effects on IOP and PD in eyes contralateral to the side in which unilateral cervical ganglionectomy had been performed. If crossover NE supersensitivity is indeed present, the contralateral eye is not a true control following unilateral cervical ganglionectomy. We therefore elected to do our studies with bilaterally ganglionectomized (BG) animals to avoid the problem of crossover supersensitivity and to enhance the response. Because of our experimental design, the statistical results we report take into account the variance between rabbits and between eyes.

Our research indicates that a single topical application of NE elicits an early hypertonic IOP response in conscious rabbits—an effect which is reversed by pretreatment with topical indomethacin. The corresponding mydriasis could be significantly reduced by phenoxybenzamine (PBA) but not by indomethacin.

Methods
Male New Zealand albino rabbits (2 to 3 kg) were used in all studies and were acclimated to laboratory conditions for 1 week. During the subsequent 2 weeks, the animals were "trained" by twice-weekly IOP and PD measurements so that they required only gentle hand restraint. IOP measurements were made with a Mackay-Marg No. 12 tonometer after applying 1 drop of 0.5% Pontocaine to each eye. Pupil diameters were measured in a windowless room with overhead lighting of constant intensity. The PD measurements were made with a metric ruler, with the canthi used as reference points.

All solutions of drugs were made with water which was boiled and then bubbled with argon while cooling. The drug doses used were concentrations found by others to be effective with respect to the parameters (IOP and PD) being measured.

In some of our early studies, NE in the form of the HC1 salt (L-arterenol • HC1, Sigma Chemical Co., St. Louis, Mo.) was applied topically to both eyes of each animal as 50 μl of a 0.1M solution. However, most studies used NE in the form of the bitartrate salt (L-arterenol bitartrate, Sigma) at doses of 0.1, 5, 12 and 50 μmol applied to both eyes as 50 μl aliquots of 0.002M, 0.1M, and 1.0M solutions, respectively. The NE was stored at 4° C for up to 1 week as a 0.5M stock solution. This NE stock solution was diluted with water to the desired concentration just prior to use.

PBA ampules containing 100 mg/dl PBA per 2 ml were diluted to 50 ml with 0.9% NaCl and injected 90 min prior to IOP and PD determinations. When PBA treatment was indicated, rabbits were given a total of 1 mg/kg body weight by injection into the lateral ear vein.

Ganglionectomy was accomplished by bilateral sympathectomy anterior to the superior cervical
Fig. 2. Bars represent mean ± S.E. of IOP. Dotted bars, NO-OP (54 eyes of 27 animals). Cross-hatched bars, SHAM (48 eyes of 24 animals); solid bars, BG (50 eyes of 25 animals). Opened portions of the bars and statistical comparisons as in legend to Fig. 1. IOP was measured 90 min after administration of PBA (when used) and 30 min after administration of NE. PBA and NE were administered as described in the text. The p values after PBA administration (vs. Before) for the NO-OP, SHAM, and BG animals are <0.001, <0.001, and <0.05, respectively. For post-NE vs. PBA pretreatment, the p values are <0.005, <0.005, and NS, respectively. For NE vs. Before (no PBA pretreatment), the p values are <0.001, <0.001, and <0.01, respectively.

ganglia, with subsequent removal of the nerve segment containing the cervical ganglia. The postoperative drug studies on these animals were begun 2 to 3 weeks after surgery.

Groups 1 through 5 referred to below each represent a grouping of bilateral superior cervical ganglionectomized (BG), sham-operated (SHAM), and nonoperated (NO-OP) rabbits. Group 1 consisted of 7, 6, and 9 BG, SHAM, and NO-OP rabbits, respectively; group 2 consisted of 9, 9, and 8 BG, SHAM, and NO-OP rabbits, respectively; group 3 consisted of 9, 9, and 10 BG, SHAM, and NO-OP rabbits, respectively; group 4 consisted of 8, 10, and 8 BG, SHAM, and NO-OP rabbits, respectively; and group 5 consisted of 10, 10, and 8 BG, SHAM, and NO-OP rabbits, respectively.

The data from the first three groups (25 BG rabbits, 24 SHAM rabbits, and 27 NO-OP rabbits) were pooled and used to examine the IOP and PD responses to topical NE at 30 min after the drug (data of Figs. 1 and 2). The ability of previously administered intravenous PBA to antagonize these NE-induced effects was also studied with these same three groups.

The fourth group of rabbits was used to determine the early time course of the IOP and PD responses to single topical applications of 0.1, 5, and 50 μmol NE (data of Figs. 3 to 5).

The fifth group of rabbits was used to determine the effect of indomethacin pretreatment upon the time course of response to NE (data of Fig. 6). Topical indomethacin (50 μl of a 1% solution in sesame oil), when used, was given at 3, 2, 1, and 0.5 hr prior to NE administration. This is a dose shown to be effective in other studies in both man and rabbit.

Statistical tests were made by the Student t test for paired or unpaired groups. Experimental values are expressed as the arithmetic mean ± the standard error.

Results

Effect of NE on IOP and PD after 30 min.

When NE (5 μmol/eye) was applied topically, there was a significant increase in pupil diameter when compared to before-drug measurements (Fig. 1). The mydriatic response of the BG animals also showed a significant NE supersensitivity (BG vs. SHAM or NO-OP, p < 0.001). Although NE slightly but significantly increased IOP at 30 min, the comparison of IOP measurements for BG vs. SHAM or NO-OP (unpaired Student t test) gave no evidence of NE supersensitivity for this parameter at this time interval (Fig. 2).

When the animals were pretreated with intravenous PBA, the mydriatic response to subsequent treatment with topical NE was significantly less than in the absence of PBA pretreatment (p < 0.001) within the NO-OP, SHAM, and BG animals (Fig. 1, open bars). PBA pretreatment also significantly reduced the IOP (Fig. 2). The magnitude of PBA effect on the BG rabbits was less than on the controls, but even after subsequent NE application, the IOP values of BG animals were still significantly reduced with respect to before-drug measurements (p < 0.025). Following PBA pretreatment, the BG rabbits had IOP values significantly higher than did the SHAM or NO-OP animals, both before (p < 0.001) and after (p < 0.001) NE treatment (unpaired t test) (Fig. 2).

When changes in IOP produced by NE (no PBA pretreatment) with respect to before-drug (Fig. 2, open bars above NE) were com-
pared with changes in IOP produced by PBA plus NE (Fig. 2, filled-in portion of bars above NE), there was a significant drop in the IOP of NE-treated rabbits. This drop was attributable to PBA antagonism of NE effects (p < 0.001, paired t test, in NO-OP vs. NO-OP, SHAM vs. SHAM, and BG vs. BG).

Regarding baseline comparisons of IOP, there was a significantly greater IOP for all BG animals (Fig. 2) when compared with all SHAM and NO-OP measurements before drugs (p < 0.001, unpaired t test).

**Time-course studies of NE on IOP and PD**

**Effects on IOP (0 to 60 min after NE).** Except in the case of indomethacin pretreatment, the IOP (paired t test for NO-OP vs. NO-OP, SHAM vs. SHAM, and BG vs. BG) was always significantly elevated (p < 0.005) 10 min after application of topical NE (Figs. 3 to 6, bottom). With 5 \( \mu \)mol NE per eye in the absence of indomethacin, the BG rabbits had a 7.5 mm Hg increase in IOP (compared to predrug values) 10 min following drug administration. At 60 min after application of 5 or 50 \( \mu \)mol/eye, the BG animals no longer showed elevations of IOP significantly different from their own baseline values (Figs. 4 and 5, bottom).

After topical administration of 5 \( \mu \)mol NE per eye (the dose used in the indomethacin pretreatment studies described below), applying the data from Fig. 4, we used an unpaired Student t test analysis to compare the IOP of control animals with that of the BG animals. In the NO-OP–BG comparisons before NE administration and at 10, 15, 20, 30, and 60 min after the drug, the p values were <0.05, <0.001, <0.005, <0.05, NS (not significant), and NS, respectively. In the SHAM-BG comparisons at the same time intervals, the p values were <0.005, <0.005, <0.001, <0.05, NS, and NS, respectively.

At the lowest dose of NE (0.1 \( \mu \)mol/eye), the IOP of BG rabbits was significantly
greater than that of the combined SHAM and NO-OP animals at 10 and 30 min, but there were no significant differences between the IOP values of these combined controls vs. BG at 60 min (Fig. 3, bottom). With the two higher doses of NE, the peak elevation in IOP occurred earlier in the BG animals and was significantly greater than in controls 10 min after drug administration (Figs. 4 and 5, bottom). However, at 30 min with the two higher doses, the IOP values of BG animals were not significantly different from those of SHAM and NO-OP animals (Figs. 4 and 5, bottom). This latter result is in agreement with the results in Fig. 2 for the previous studies with the 5 μmol NE dose.

At the highest dose of NE (50 μmol/eye), the IOP of the BG rabbits dropped significantly lower than that of the combined controls after 60 min (Fig. 5, bottom). In contrast to the significant elevation in IOP observed 10 min following topical NE in the absence of indomethacin, pretreatment of the BG animals with topical indomethacin resulted in a significant drop in IOP 10 min after 5 μmol NE per eye were administered (p < 0.001) (Fig. 6, bottom).

In the studies involving pretreatment with indomethacin followed by topical administration of 5 μmol NE per eye, applying the data from Fig. 6, we used an unpaired Student t test analysis to compare the IOP of control animals with that of the BG animals. In the NO-OP–BG comparisons before indomethacin, at zero time (just prior to NE administration) and 10, 15, 20, 30, and 60 min post-NE treatment, the p values were <0.05, <0.001, NS, NS, NS, <0.01, and <0.001, respectively. In the SHAM–BG comparisons at the same time intervals, the p values were <0.025, <0.005, NS, NS, NS, NS, and <0.005, respectively.
As was found with the earlier studies (Fig. 2), the baseline IOP data (values recorded just prior to NE treatment) in the time-course study were, with one exception, slightly but significantly higher in the BG animals when compared with either of the controls or with the controls combined (Figs. 3 to 5, bottom). This same pattern of IOP elevation in BG animals relative to SHAM and NO-OP animals was seen again prior to topical NE when we examined the effect of indomethacin treatment (Fig. 6, bottom).

No significant difference was found between left and right eyes when the IOP data from either the BG or the control animals of group 4 (extracted from the data used in Figs. 3 to 5, bottom) were examined statistically at each of the time intervals. Analysis of variance in the same group also revealed no significant difference between BG animals and each of the control groups for all time intervals.

Effects on PD (0 to 60 min after NE). The top half of Figs. 3 to 6 shows the effect of NE on PD for the time-course studies with and without indomethacin pretreatment. It was noted that there was a significant (p < 0.001) and sustained mydriasis 10 min following administration of NE at all three doses. At the highest dose of NE (50 μmol/eye), the controls (SHAM and NO-OP) showed increasing mydriasis until the end of the experiment, but maximum pupil dilation of controls was reached at 30 and 10 min for the 5 and 0.1 μmol/eye doses, respectively. The BG rabbits exhibited significantly greater PD than the controls (p < 0.001) at all times following each of the three doses of NE. The time course of mydriasis for BG animals at a dose of 0.1 μmol/eye (Fig. 3, top) can be compared closely with the time course of PD for the SHAM and NO-OP animals which received 50 μmol/eye (Fig. 5, top), indicating that the BG animals had become quite supersensitive.

By comparing Figs. 4 and 6, top, it is seen that topical pretreatment with 1% indomethacin in sesame oil had no appreciable effect upon the PD response to NE (5 μmol/eye).

The time course for the effects of 5 μmol L-arterenol · HCl upon IOP and PD of NO-OP rabbits was determined in the preliminary phase of these experiments and was found not to differ significantly from the same concentration of bitartrate salt.

Discussion

Effects of NE on IOP and PD. An elevation of IOP above predrug levels as great as 7.5 mm Hg as early as 10 min following an initial topical application of NE has not, to our knowledge, been reported previously. Although elevated IOP values were observed with the control groups in our studies, particularly at a dose of 50 μmol/eye, BG exaggerated the effects and changed the time course, indicating catecholamine supersensitivity in the BG animals. Although control groups showed a peak elevation of IOP at 20 to 30 min, the BG rabbits exhibited maximal responses during this latter time period only with the lowest dose of NE (0.1 μmol/eye). As the dose of NE was increased, the peak response was accelerated to as early as 10 min in the same BG animals. The same increased amount of NE also accelerated the subsequent drop in IOP. With a NE dose of 5 μmol/eye for animals of groups 1, 2, and 3, we observed a significant difference in the changes relative to before-drug levels for the 30 min post-NE IOP response when the same animals were compared with and without PBA pretreatment. We suppose that the 10 to 15 min NE-induced increase in IOP observed with the animals of group 4 may also be mediated by α-receptor function, but this α-receptor function remains to be tested at that time interval. Data from several other sources would corroborate such a function. Langham and Palewicz report that PBA blocked a 1 to 4 mm Hg hypertensive IOP response which they measured at 60 min following multiple doses of topical NE. The significant difference between IOP values of BG and control groups following PBA pretreatment may indicate an incomplete block of the supersensitive BG rabbits at the dosage of PBA used here.

Aside from the fact that significant early IOP responses (prior to 60 min after NE ad-
ministration) have not been reported, it may be that any excitement which rabbits experience, such as might be induced by constraint in canvas bags, 1-4, 16 would tend to obscure the early response we have observed. Current concepts of the way in which presynaptic receptor systems control catecholamine (synaptic release) would suggest that circulating epinephrine can cause such modulation of the response. 17

In the absence of NE treatment, our data show a significantly higher IOP in eyes of BG rabbits at baseline conditions (compared with both control groups), and this elevation persists following indomethacin pretreatment. Such a persistent elevation has not been reported, since very few studies have been made of BG rabbits. We postulate that a constant or tonic release of endogenous NE may normally reduce IOP slightly by a mechanism similar to that of the 3 to 4 hr response to topical NE. 1-3 If this postulate is true, then it would seem likely that the increased IOP we observed in the BG rabbits reflects the absence of this tonic release of NE from adrenergic nerve endings which are no longer present.

In the experiments reported here, the elevated IOP response following topical NE had a similar or slightly earlier latency than the PD response. This is in sharp contrast to the delayed decrease in IOP which has been reported by others and which occurs following the peak PD response at a time 3 to 6 hr after NE treatment. 1-3 Such differences in response suggest dissimilar mechanisms of action.

Part of the early rise in IOP probably results from NE-stimulated PG synthesis, since indomethacin (an inhibitor of PG synthesis 18) reversed this response in BG animals. Also, it has been shown that a low level of PG can reduce IOP in normal rabbits. 7 It would follow, then, that we cannot be certain that our indomethacin pretreatment completely blocks PG production. The reason for the failure of indomethacin pretreatment to reverse the NE-induced IOP increase in the NO-OP and SHAM animals is not clear. PG synthesis and release are general phenomena following NE treatment of nonocular tissues, 19-21 and, as implied by our results, it is apparently also synthesized when NE is topically applied to the eye. Such a conclusion has been reached by Bengtsson 22 relative to studies of effects of PGE2 and adrenergic agents on aqueous flare. As pointed out by Starke et al, 17 extensive evidence has been gathered to support a general mechanism whereby a postsynaptic formation of PGs during noradrenergic transmission acts through local synaptic feedback, and the PGs so formed depress further presynaptic release of NE. However, NE is also said to inhibit its own further release by activation of presynaptic α-adrenoreceptors 23.

In our laboratory, a 30 sec in vitro incubation of ciliary process tissue with NE was found to cause a greater formation of PGs and PG metabolites than similar incubations with isoproterenol (Woods, unpublished observations).

Sympathetic nerve stimulation also increases the formation and release of PGs. 24-29 This formation of PGs probably occurs at postsynaptic sites, since it is blocked by PBA. 24, 30 Direct measurements of PG formation during in vivo treatment of the eye with NE have not been made, but indomethacin has been reported to reduce the hyperemia of the iris observed during the release of NE after superior cervical ganglionectomy. 31 Sears 10 states that “release of prostaglandins is dependent on the presence or release of norepinephrine because pretreatment with an inhibitor of norepinephrine biosynthesis, α-methyltryosine, also prevents the hyperemia.” Neufeld and Page 32 also proposed that the enhancement of NE release by indomethacin during field stimulation of adrenergic nerves of the iris is mediated by PGs.

Intraocular injection of PGs is known to induce miosis. 11, 33, 34 If NE induces endogenous PG synthesis or release 16, 31 in the iris, we should have observed a further increase in PD for the SHAM and NO-OP eyes following indomethacin pretreatment (less PG synthesis and resultant miosis). Since a further increase in PD was not found, it is apparent
that any NE-stimulated PG synthesis occurring in iris tissue has a minimal effect on PD. We conclude that NE plays a more direct and predominant role upon PD than upon the hypertensive response (significantly blocked by indomethacin pretreatment) to NE at 10 to 15 min post-NE treatment.

The fact that pretreatment with indomethacin enhanced the hypotensive response to NE suggests a more direct relationship between NE and the lowering of IOP than is seen for the hypertensive response.

The excellent technical assistance of Miss Mary Louise Law and Mr. Michael Barron is acknowledged with thanks. We thank the Smith Kline & French Laboratories for our supply of phenoxybenzamine.

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