Antagonism of Formaldehyde-Induced Ocular Hypertension by Phenylethylamines (35030)

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Topical administration of formalin has been shown to produce ocular hypertension accompanied by miosis and vasodilatation in rabbits (1). These symptoms are similar to those observed following irritation to the eye by other chemicals or mechanical means (2–5). The ocular response to formaldehyde may be due to irritation of extraocular tissues and/or intraocular tissues. It was of interest to determine the ocular response to intracamerally-infused formaldehyde.

Since isoproterenol and epinephrine have been shown to antagonize the ocular hypertensive response to topically administered formaldehyde (1), their effects on the ocular hypertensive response to intracamerally-infused formaldehyde were subsequently tested. The effects of norepinephrine and phenylephrine were also studied in the present experiment.

Materials and Methods. Albino rabbits of either sex weighing 1.5–2.5 kg were used. In the control group some animals received 2 drops of 0.9% saline approximately 60 min before the formaldehyde infusion. In the experimental groups animals were pretreated topically with 2 drops of isoproterenol, epinephrine, norepinephrine, or phenylephrine approximately 30 or 60 min before the formaldehyde infusion. Approximately 20 min before the formaldehyde infusion animals were anesthetized with 1–2 g/kg of urethane intravenously via the marginal ear vein as a 25% solution in 0.9% saline, and placed in a prone position. The anterior chamber of one eye was cannulated at the 12 o'clock position with a 22-gauge needle connected by a polyethylene tubing No. 50 (PE 50) to a Statham P 23 Db pressure transducer. The needle and PE 50 tubing had been filled with 0.2% formaldehyde before cannulation. The transducer was connected through PE 260 tubing to a distilled water reservoir set at 25 mm Hg height, a 2-ml syringe on a Harvard infusion pump, and an outlet tubing for zero level adjustment. The changes in the intraocular pressure (IOP) were recorded on a Beckman Type RB Dynograph. The sensitivity of the recorder was set at 1 mm Hg/mm scale and the paper speed at 1 cm/min.

Immediately after cannulation the needle was connected to the transducer and the infusion pump by turning off the connection to the reservoir. The pressure in the transducer fell from 25 mm Hg to the basal IOP level within 15 min. As soon as the IOP was constant (less than 1 mm Hg change in the IOP within 3-min period) the infusion was begun at a speed of 1.97 μl/min. In the control group the duration of infusion was varied from 1 to 4 min. In the drug pretreated groups the duration of infusion was 3 min. The IOP was continuously monitored during the infusion and for a period of 10 min after the termination of infusion.

The 0.2% formaldehyde solution was prepared twice a day by diluting 0.27 ml of 36.8% formaldehyde solution (Formaldehyde solution, Fisher) with 0.9% saline to 50 ml. Solutions of *l*-isoproterenol *d*-bitrartrate, *l*-epinephrine *d*-bitartrate, *l*-norepinephrine *d*-bitrartrate and *dl*-phenylephrine hydrochloride were prepared freshly in 0.9% saline. Statistical analysis was performed according to the Student's *t* test (6). A *p* value of less than .05 was considered as significant.

Results. The infusion of formaldehyde into the anterior chamber of urethane-

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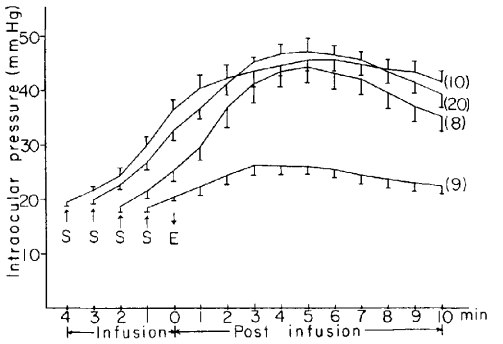

FIG. 1. The effect of formaldehyde infusion on the intraocular pressure of urethane anesthetized rabbit. 0.2% Formaldehyde was infused into the anterior chamber of the rabbit eye at a rate of 1.97 μl/min for either 1, 2, 3, or 4 min. Infusion starts at S (↑) and ends at E (↓). The intraocular pressure was continuously monitored during the 10-min postinfusion period. The mean intraocular pressure with its standard error is shown at each point. The number of animals in each group is given in parentheses.

anesthetized rabbits consistently produced miosis, vasodilatation, and ocular hypertension. Only the latter was measured in the present experiment. Figure 1 summarizes the ocular hypertensive response to intracameral infusion of formaldehyde. The infusion of 0.2% formaldehyde for 1 min (equivalent to 3.94 μg or 0.13 μmole of formaldehyde) produced a significant elevation in the IOP during the 10-min postinfusion period. With the increase in the duration of infusion the IOP went up even higher. In fact, the IOP started to go up during the infusion when the period of infusion was longer than 2 min. The infusion of 0.9% saline under similar conditions only raised the IOP to a new steady IOP (approx 10 mm Hg above the basal IOP) which returned to its basal IOP during the postinfusion period. Since the 3-min infusion produced the maximal response it was adopted as the standard procedure for the drug-treated groups.

The effect of formaldehyde infusion in one eye on the IOP of the other eye was studied in some animals by recording the IOP of both eyes simultaneously. The pressures of these eyes were continuously monitored for 25 to 50 min. Infusion of formaldehyde for 1 min produced a maximal elevation of 1.6 mm Hg (av of 0, 0, 0.6, and 5.8 mm Hg) in the other noninfused eye. Three- and 4-min infusion produced an elevation of 2.6 mm Hg (av of 2.0 and 3.2 mm Hg) and 8.4 mm Hg (av of 3.6, 4.8, and 16.8 mm Hg), respectively, in the other noninfused eye. The maximal elevation in the IOP of the infused eye always occurred within 7 min after the termination of infusion while the maximal elevation in the IOP of the other noninfused eye occurred between 12 and 37 min after the termination of infusion. No change in femoral arterial blood pressure was observed in two rabbits during and after the intracameral infusion of formaldehyde.

To determine the effects of isoproterenol, epinephrine, norepinephrine, or phenylephrine on the response to formaldehyde infusion, one eye of each animal was pretreated topically with 2 drops of one of these four phenylethylamines. The results on the basal IOP and the maximal change in the IOP during the postinfusion period are shown in Table I. Topical pretreatment with 0.9% saline for 60 min did not affect these parameters. Pretreatment with isoproterenol resulted in a reduction in the response to formaldehyde. There were two dose–response relationships: one at doses between 0.003 and 0.055 *M* and another at doses higher than 0.055 *M*. The basal IOP was also lowered by isoproterenol at concentrations which did not affect the ocular hypertension following the formaldehyde infusion. The effect of 60-min pretreatment with isoproterenol was more pronounced than that of 30-min pretreatment. The effects of pretreatment with epinephrine or norepinephrine were qualitatively similar to those of isoproterenol. There was a dose–response relationship within the concentrations tested. Phenylephrine also lowered the basal IOP and prevented the response to formaldehyde at a concentration of 0.44 *M* or higher. However, 0.88 *M* solution was no more effective than 0.44 *M* solution. It is interesting to note that isoproterenol was the most active agent in lowering the basal IOP and that epinephrine was the most active agent in preventing the ocular hypertensive

TABLE I. Effects of Topically Administered Isoproterenol, Epinephrine, Norepinephrine, or Phenylephrine on Formaldehyde-Induced Ocular Hypertension in Rabbits.

Pretreatments				
Substance	Time (min)	No. of expts.	Basal IOP (mm Hg)	Maximal changes in IOP (mm Hg)
None		20	20.0 ± 0.8[a]	28.4 ± 1.3
0.9% Saline	60	10	20.5 ± 0.6	28.4 ± 2.1
Isoproterenol				
0.055 *M*	30	6	15.8 ± 0.9	27.1 ± 2.8
0.003 *M*	60	6	16.8 ± 0.8	27.8 ± 3.8
0.007 *M*	60	10	15.9 ± 0.7	19.5 ± 2.1
0.027 *M*	60	12	16.2 ± 0.5	17.5 ± 2.9
0.055 *M*	60	9	15.9 ± 0.5	17.6 ± 3.2
0.110 *M*	60	6	15.4 ± 0.8	11.1 ± 1.6
Epinephrine				
0.055 *M*	30	6	17.2 ± 1.3	16.8 ± 3.2
0.020 *M*	60	7	17.0 ± 0.8	21.3 ± 1.8
0.039 *M*	60	8	17.8 ± 0.4	12.7 ± 2.8
0.055 *M*	60	7	16.2 ± 0.8	6.8 ± 1.0
Norepinephrine				
0.110 *M*	30	6	17.0 ± 1.1	18.0 ± 2.7
0.027 *M*	60	6	18.4 ± 0.9	23.6 ± 2.8
0.055 *M*	60	10	18.8 ± 0.6	18.0 ± 2.7
0.110 *M*	60	8	16.1 ± 0.5	11.0 ± 1.5
Phenylephrine				
0.110 *M*	60	6	18.4 ± 0.4	28.5 ± 3.1
0.440 *M*	60	5	18.6 ± 0.6	16.4 ± 2.1
0.880 *M*	60	5	17.1 ± 0.8	18.3 ± 3.6

[a] Mean ± SE.

effect of formaldehyde.

Discussion. Intracameral infusion of formaldehyde consistently produced miosis, vasodilatation, and ocular hypertension in urethane-anesthetized rabbits. The ocular hypertensive effect was dose dependent, and was maximal within 7 min after the termination of infusion. These results are consistent with those reported previously from this laboratory using the topical administration of 2.5% formalin (1). The ocular responses to formaldehyde are identical to those reported by others using other chemical or mechanical means (2–5). A small and delayed increase in the IOP occurred in the other noninfused eye. This is consistent with the data reported by others (3, 7). It has been suggested that prostaglandins are involved in the response of eye to chemical or mechanical stimuli (8–10). It is possible that prostaglandins also mediate the ocular hypertensive response to formaldehyde infusion.

The effects of phenylethylamines in antagonizing the ocular response to chemical or mechanical stimuli (1, 4) or prostaglandins (11) have been well documented. According to the currently accepted theory of adrenergic receptors (12) the order of decreasing activity of the beta adrenergic receptor stimulation among the four compounds tested is: isoproterenol, epinephrine, norepinephrine, and phenylephrine. The same order of activity holds for the increasing activity of the alpha adrenergic receptor stimulation. Alpha adrenergic receptor stimulation has been implicated in the increase in aqueous outflow and beta adrenergic receptor stimulation in the decrease in aqueous formation (13, 14). Pretreatment with phenylephrine, a typical alpha adrenergic receptor agonist, antagon-

ized the formaldehyde effect. However, the effect of phenylephrine did not increase with the increase in dose. The antagonistic effects of epinephrine or norepinephrine increased continuously with the increase in doses. The dose–response curve for isoproterenol, a typical beta adrenergic receptor agonist, had two phases. The initial phase occurred at doses lower than 0.055 *M* and the second phase at doses higher than 0.055 *M*. Large doses of isoproterenol have been shown to produce an alpha effect on the pupil and outflow mechanism (14). The present data suggest that the antagonistic effects of phenylethylamines on the ocular hypertensive response to formaldehyde are due to the stimulation of alpha and/or beta adrenergic receptors in the eye. The effect of alpha or beta adrenergic receptor stimulation may operate independently to a plateau level of approximately 40% inhibition of the formaldehyde response as demonstrated by phenylephrine and low doses of isoproterenol, and synergize with each other in the cases of epinephrine, norepinephrine, and high doses of isoproterenol.

Summary. Intracamerally infused formaldehyde produced miosis, vasodilatation, and ocular hypertension similar to those produced by topically applied formaldehyde. A small, delayed ocular hypertension was also observed in the other noninfused eye. Topical administration of isoproterenol, epinephrine, norepinephrine, or phenylephrine prevented the ocular hypertensive response to intracamerally infused formaldehyde.

1. Fortenberry, B., Kagawa, C. M., and Leaders, F. E., Jr., Proc. Soc. Exp. Biol. Med. **131**, 637 (1969).

2. Duke-Elder, P. M., and Duke-Elder, W. S., Proc. Roy. Soc., Ser. B. **109**, 19 (1931).

3. Perkins, E. S., Brit. J. Ophthalmol. **41**, 257 (1957).

4. Thomas, R. P., Amer. J. Ophthalmol. **65**, 729 (1968).

5. Sears, M. L., Arch. Ophthalmol. **63**, 707 (1960).

6. Snedecor, G. W., "Statistical Methods." Iowa State Univ. Press, Ames (1957).

7. Davson, H., and Matchett, P. A., J. Physiol. (London) **113**, 387 (1951).

8. Ambache, N., Kavanagh, L., and Whiting, J., J. Physiol. (London) **176**, 378 (1965).

9. Waitzman, M. B., and King, C. D., Amer. J. Physiol. **212**, 329 (1967).

10. Beitch, B. R., and Eakins, K. E., Brit. J. Pharmacol. **37**, 158 (1969).

11. Waitzman, M. B., Amer. J. Physiol. **217**, 1593 (1969).

12. Ahlquist, R. P., Amer. J. Physiol. **153**, 586 (1948).

13. Eakins, K. E., J. Pharmacol. **140**, 79 (1963).

14. Eakins, K. E., and Ryan, S. J., Brit. J. Pharmacol. **23**, 374 (1964).

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