

Effect of propranolol on the isoproterenol, responses of cortisol, isoproterenol, and aminophylline

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We have studied the effect of propranolol on the eosinopenic responses of cortisol, isoproterenol, and aminophylline. Blood was drawn for a base-line total eosinophil count (TEC) from fasting male volunteers at 8 A.M. followed by propranolol, 40 mg., or a placebo orally. Later, one drug or a placebo was given according to the following schedule: cortisol, 25 mg. intravenously at 9 A.M., isoproterenol, 0.05 mg. subcutaneously at 11 A.M., and aminophylline, 500 mg. intravenously at noon. A second base-line TEC was obtained at 11 A.M., and a final TEC was obtained in all patients at 1 P.M. When only placebos were given, a significant mean fall in the TEC of 14 per cent was seen in the first 3 hours ($p < 0.001$), followed by no further significant change in the remaining 2 hours. After propranolol followed by placebos, a similar fall in the TEC was observed at 3 hours. Mean values for the change in TEC after isoproterenol alone was -34% ($p < 0.01$), after aminophylline alone, -23% ($p < 0.005$), and after cortisol alone, -68% ($p < 0.01$). When preceded by propranolol, the mean change in TEC was not significant after isoproterenol and aminophylline (+1 per cent and +8 per cent respectively) and remained significant after cortisol, -56% ($p < 0.05$).

The eosinopenic effect of corticosteroids and epinephrine has long been recognized, although the mechanism of their action remains poorly understood. There is evidence that corticosteroids cause a decreased release of eosinophils from the marrow.¹ A direct lytic action of corticosteroids on the eosinophil is unlikely, but increased peripheral sequestration has not been completely ruled out.²

Less is known about the eosinopenic effects of epinephrine. The initial impression that its action was mediated by activation of the anterior pituitary-adrenocortical axis³ has been discounted, although a certain minimum level of adrenocortical activity does appear to be necessary.⁴ The observation that propranolol will selectively block the eosinopenic effect of epinephrine suggests that activation of beta-adrenergic receptors is involved in its action.⁵ The reduced eosinopenic effect of epinephrine in asthmatic patients is evidence for a beta-adrenergic blockade in these patients.⁶

The possibility that activation of beta-adrenergic receptors is a factor in the eosinopenic effect of corticosteroids has not been excluded. Furthermore, amino-

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TABLE I. Experimental protocol

Time	All subjects	Schedule for administration of cortisol, isoproterenol, or aminophylline
8 A.M.	Initial base-line blood sample; 40 mg. of propranolol or a placebo orally	
9 A.M.		25 mg. of cortisol intravenously
11 A.M.		Base-line blood sample prior to administration of isoproterenol or aminophylline; 0.05 mg. of isoproterenol or a placebo subcutaneously
12 noon		500 mg. of aminophylline or a placebo intravenously
1 P.M.	Final blood sample	

phylline, a drug that, like epinephrine, increases levels of cellular cyclic adenosine monophosphate (AMP), has not been studied in reference to its action on the eosinophil count.

We have therefore studied the effect of propranolol pretreatment on the total eosinophil count (TEC) as modified by cortisol, isoproterenol (a selective beta-adrenergic stimulator), and aminophylline.

METHODS AND EXPERIMENTAL PROTOCOL

Eosinophils were counted in Fuch-Rosenthal chambers as described previously,⁷ using coded samples of venous blood with ethylenediaminetetraacetic acid (EDTA) as an anticoagulant. Ten consecutive measurements on one such sample yielded a mean count of 87 per cubic milliliter, with a standard error of the mean of 2.4 per cubic millimeter.

Healthy male volunteers between the ages of 18 and 32 years who had no history of allergic disease were selected. After an 8 hour fast, venous blood samples were drawn for base-line eosinophil counts at 8 A.M.* The volunteers then received either a placebo or 40 mg. of propranolol orally, whereupon cortisol, isoproterenol, or aminophylline was administered as follows: 25 mg. of cortisol intravenously at 9 A.M., 0.05 mg. of isoproterenol subcutaneously at 11 A.M., or 500 mg. of aminophylline intravenously at 12 noon. A second base-line blood sample was drawn at 11 A.M., prior to the administration of isoproterenol or aminophylline. Prior to and 10 minutes following the administration of isoproterenol, the heart rate was measured using the radial arterial pulse. In addition, subcutaneous and intravenous placebos were given at 11 A.M. and 12 noon, respectively, to test the possible placebo effect of these injections. Propranolol alone was administered to another group, followed by a subcutaneous placebo at 11 A.M. in order to test the effect of propranolol on the TEC. A final blood sample was drawn at 1 P.M. in all subjects. This was 5 hours after the oral administration of propranolol or a placebo. These intervals were chosen to include the maximum eosinopenic effects of the drugs under study within the time of propranolol's pharmacologic action and to reach termination of the period of study of each of the 3 drugs at the same time. The experimental protocol is summarized in Table I.

RESULTS

Cortisol, isoproterenol, and aminophylline all produced significant decreases in the TEC over the time intervals studied. The effect of pretreatment with propranolol and the statistical analysis are summarized in Table II.

*Subjects who had 8 A.M. total eosinophil counts of less than 50 per cubic millimeter were excluded from the study.

TABLE II. The effect of propranolol on the eosinopenic responses of cortisol, isoproterenol, and aminophylline

	Cortisol (25 mg. intravenously at 9 A.M.)		Isoproterenol (0.05 mg. subcutaneously at 11 A.M.)		Aminophylline (500 mg. intravenously at 12 noon)	
	After oral placebo	After oral propranolol	After oral placebo	After oral propranolol	After oral placebo	After oral propranolol
Mean base- line TEC*	153 ± 70† (7)	251 ± 243 (8)	149 ± 57 (9)	122 ± 21 (8)	203 ± 117 (9)	143 ± 53 (7)
Mean final TEC (1 P.M.)	47 ± 28	108 ± 93	95 ± 36	122 ± 27	162 ± 121	158 ± 72
p value (paired t test)	< 0.01	< 0.05	< 0.01	NS‡	< 0.005	NS
Mean per cent change in TEC	-68	-56	-34	+1	-23	+8

*Blood was drawn at 8 A.M. prior to administration of cortisol and at 11 A.M. prior to administration of isoproterenol, or aminophylline.

†One standard deviation.

‡Number in the group.

§Not significant.

Following an oral placebo, 25 mg. of cortisol intravenously produced a mean fall in the TEC of 68 per cent (Fig. 1). After 40 mg. of propranolol orally, the same dose of cortisol produced a 56 per cent mean decrease, which is not significantly different from the effect of cortisol alone. It is of interest that, following a placebo in place of propranolol alone over the same time interval from 8 A.M. to 1 P.M., there was a small but highly significant mean decrease of 16 per cent ($p < 0.001$). This fall was significantly less than that produced by cortisol, whether preceded by propranolol or not. We have, like others,³ observed this spontaneous decrease in the TEC in a much larger group of patients. It occurred between the hours of 8 A.M. and 11 A.M. and was not affected by pretreatment with propranolol (Table III). From 11 A.M. to 1 P.M. there was a spontaneous tendency for the TEC to rise, but this was not significant. The changes in the TEC following propranolol alone did not differ from those following a placebo in any of the time intervals studied. Following an oral placebo at 8 A.M. and a subcutaneous placebo at 11 A.M., during the time interval from 11 A.M. to 1 P.M. the TEC changed by a mean +13 per cent (not significant) in 9 subjects. During the same interval following 40 mg. of propranolol orally at 8 A.M. and a subcutaneous placebo at 11 A.M., the TEC changed by a mean +10 per cent in 10 subjects, which is not significantly different.

The effect of administering 0.05 mg. of isoproterenol subcutaneously is illustrated in Fig. 2. When preceded by the oral placebo, isoproterenol produced a significant mean decrease in the TEC of 34 per cent over 2 hours. Pretreatment with propranolol completely blocked this effect. A subcutaneous placebo in place of isoproterenol had no significant effect (+13 per cent) on the TEC over the same 2 hour interval. The mean radial pulse rose from 72 per minute to 99 per minute 10 minutes following isoproterenol administration respectively ($p < 0.01$). Propranolol pretreatment completely blocked this effect, with correspond-

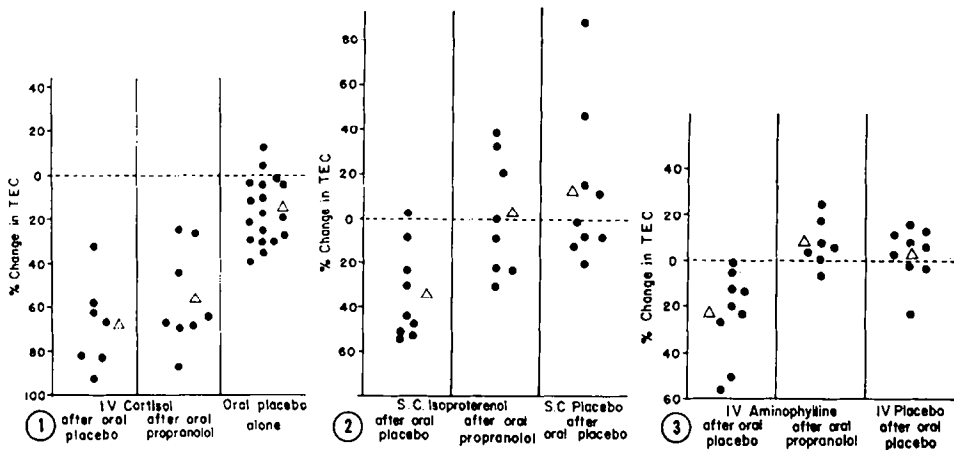


FIG. 1. The effect of propranolol on the eosinopenic response of cortisol. Forty milligrams of propranolol or a placebo was given orally at 8 A.M., and 25 mg. of cortisol was given intravenously at 9 A.M. The change in the total eosinophil count from 8 A.M. to 1 P.M. was measured.

FIG. 2. The effect of propranolol on the eosinopenic response of isoproterenol. Forty milligrams of propranolol or a placebo was given orally at 8 A.M., and 0.05 mg. of isoproterenol or a placebo was given subcutaneously at 11 A.M. The change in the total eosinophil count from 11 A.M. to 1 P.M. was measured.

FIG. 3. The effect of propranolol on the eosinopenic response of aminophylline. Forty milligrams of propranolol or a placebo was given orally at 8 A.M., and 500 mg. of aminophylline was given intravenously at 12 noon. The change in the total eosinophil count from 11 A.M. to 1 P.M. was measured.

ing mean pulse rates of 58 per minute and 61 per minute before and after isoproterenol.

One hour after 500 mg. of aminophylline administered intravenously, the TEC fell significantly (-23 per cent) (Fig. 3). Pretreatment with propranolol completely blocked this effect. An intravenous placebo in place of the aminophylline had no significant effect (+3 per cent) on the TEC over the same one-hour interval.

The per cent decrease in the TEC following cortisol, isoproterenol, or aminophylline was examined and found to be independent of the initial base-line TEC. This has been noted before with respect to cortisol.⁸

DISCUSSION

Cortisol, isoproterenol, and aminophylline all produced a significant drop in the eosinophil count. The eosinopenic effect of isoproterenol and aminophylline but not that of cortisol was blocked by pretreatment with propranolol. This suggests that the eosinopenic effect of cortisol does not involve activation of beta-adrenergic receptors.

Any study of changes in the TEC must take into account spontaneous diurnal changes. The small but highly significant decrease in the TEC that we observed between the hours of 8 A.M. and 11 A.M. was not affected by pretreatment with propranolol, which is in keeping with the belief that this decrease is

TABLE III. Effect of propranolol pretreatment on the diurnal decrease in the total eosinophil count from 8 A.M. to 11 A.M.

	After oral placebo	After 40 mg. propranolol orally
Mean base-line TEC (8 A.M.)	202 $\pm$ 107* (35)†	178 $\pm$ 105 (25)
Mean final TEC (11 A.M.)	166 $\pm$ 77	149 $\pm$ 97
p value (paired t test)	< 0.001	< 0.01
Mean per cent change in TEC	-14	-17

*One standard deviation.

†Number in group.

caused by increased release of endogenous adrenal corticosteroids in the early morning.

It is possible that the onset of action of propranolol is sufficiently delayed so as to prevent a blocking action prior to 11 A.M. Experiments in two subjects have shown, however, that 1 hour after ingestion of 40 mg. of propranolol the chronotropic effects of isoproterenol were blocked. Thus the diurnal change in the TEC, as well as the eosinopenic effect of cortisol, likely occurred during the time of propranolol's pharmacologic action.

We have shown that isoproterenol, a selective beta-adrenergic stimulator, produced a significant decrease in the TEC that was blocked by pretreatment with propranolol. This supports the hypothesis that stimulation of beta-adrenergic receptors results in a decrease in the TEC. Previous observations that adrenalectomy abolished epinephrine-induced eosinopenia led to the belief that this effect of epinephrine was mediated by activation of anterior pituitary-adrenal cortical axis.³ Subsequent studies have shown clearly, however, that adrenal corticosteroids act in a permissive role only.⁴ The effect of beta-adrenergic stimulation on such factors as release of eosinophils from the marrow and peripheral utilization is unknown.

Aminophylline was also shown to produce a significant eosinopenia that was blocked by propranolol. This was contrary to expectations because, if the effect of aminophylline is due to decreased degradation of cyclic AMP (by inhibition of phosphodiesterase), then propranolol, which acts at the receptor level only, should not block its effect. Several explanations are possible. First, propranolol may have an effect of its own on the eosinophil count. However, when propranolol alone was administered to a group of volunteers, no significant change in the TEC was found during the time interval of aminophylline's action. Second, aminophylline might cause the secondary release of catecholamines. Although this possibility cannot be excluded, the rapidity of aminophylline's action would seem to make it less likely. Finally, and perhaps most likely, is the possibility that a certain minimum level of beta-adrenergic tone is necessary for aminophylline to act. Pretreatment with propranolol might reduce beta-adrenergic activity and intracellular cyclic AMP to a point at which aminophylline could not raise cyclic AMP sufficiently to induce an eosinopenia.

The finding of others⁵ that propranolol alone produced an increase in the TEC is difficult to reconcile with our observations. We found that the changes in the TEC from 8 A.M. to 11 A.M. and from 11 A.M. to 1 P.M. following propranolol alone did not differ significantly from the effect of an oral placebo. Our

protocol differed from that of the study referred to⁵ in that a subcutaneous placebo was administered at 11 A.M., a difference that would not seem to offer a satisfactory explanation for the discrepancy between the two studies.

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