# Antagonism of Erythropoietin Production in Rabbits by Atropine[1] (36110)

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The autonomic nervous system has been postulated to play a significant role in erythropoietin (ESF) production (1–4). Schaffer (5) reported that the destruction of the carotid sinus, aortic sinus, and sectioning of one vagus produced polycythemia in dogs which was abolished by sympathectomy. Takaku *et al.* (6) also showed that bilateral sectioning of the splanchnic nerves in rats prevented the reticulocyte response produced by renal artery constriction. Halvorsen (1) demonstrated increased ESF levels in rabbits following hypothalamic stimulation, but was unable to abolish this effect by pretreatment with phenoxybenzamine. Medado *et al.* (4) found that atropine abolished the erythropoietic response seen following hypothalamic stimulation in rats, suggesting a possible role of the parasympathetic nervous system in the control of erythropoiesis. Davis (2, 3) also reported that acetylcholinesterase produced a marked increase in reticulocytes in peripheral blood in rats and that acetylcholine produced a hyperchromic anemia in dogs.

In order to test the hypothesis that a cholinergic mechanism is involved in the control of erythropoiesis, the effects of atropine on erythropoietin production in rabbits and on the response of the polycythemic mice to ESF were studied.

*Material and Methods.* Ex-hypoxic polycythemic mice (7) were used to assay erythropoietin. HAM/ICR strain female mice (18–25 g) were made polycythemic by exposure to 0.42 atm for 22 hr each day for 2 weeks. The mice were injected subcutaneously with ½ the total dose of either saline, human urinary ESF, or rabbit plasma on days 4 and 5 following their removal from the hypobaric chamber. Each mouse received 0.5 μCi of radioactive iron ($^{59}$Fe) citrate iv on the 6th post-hypoxic day. Two days later (post-hypoxic day 8) each animal was exsanguinated via cardiac puncture and the percentage $^{59}$Fe incorporation into red blood cells (RBC) was determined.

Rabbits weighing 2.4 and 2.6 kg were exposed to 0.42 atm for either 8 or 18 hr and blood was removed via cardiac puncture for plasma ESF determination. The control rabbits and the rabbits injected with atropine sulfate (5.0 mg/kg, ip) were exposed to hypoxia (0.42 atm) for 8 or 18 hr. Immediately following removal of the rabbits from the tank their plasma was collected, heparinized, and frozen at −84° until assayed for erythropoietic activity in polycythemic mice.

In order to determine whether the dose of atropine used in the rabbits produced ganglionic blockade, the following experiments were performed: rabbits weighing between 2.5 and 2.8 kg were anesthetized with chloralose (80 mg/kg) plus sodium pentobarbital (5 mg/kg) iv. Blood pressure was recorded from the femoral or carotid artery by means of a Statham pressure transducer and a model 5 DWCB Grass polygraph. Dimethylphenylpiperazinium (DMPP, 20 μg/kg) was injected iv prior to administering atropine sulfate (5.0 mg/kg) in a single dose, and every 30 min thereafter for 5 hr.

In order to determine whether the effects of atropine on $^{59}$Fe incorporation in RBC was due to the atropine contained in the rabbit plasma sample, polycythemic mice were injected subcutaneously with ESF (0.2 units/mouse), atropine alone (5.0 mg/kg/mouse) or atropine plus ESF.

*Results.* Table I shows the plasma ESF in levels in control rabbits and rabbits pretreated with atropine prior to exposure to hypoxia (0.42 atm) for 8 hr. The mean plasma levels of ESF in 5 control rabbits (1.53 ±

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TABLE I. Plasma ESF Levels in Rabbits Exposed to Hypoxia for 8 hr.[a]

| Control | | Atropine pretreated | |
|---|---|---|---|
| Expt. no. | ESF (units/ml) | Expt. no. | ESF (units/ml) |
| 1 | 1.92 | 1 | 1.36 |
| 2 | 1.77 | 2 | 0.67 |
| 3 | 1.28 | 3 | 0.40 |
| 4 | 1.12 | 4 | 0.44 |
| 5 | 0.92 | 5 | 0.80 |
| | | 6 | 0.60 |
| | 1.53 ± 0.17 | | 0.71 ± 0.14 |

[a] ± = standard error of mean; $0.05 > p > 0.1$ (1.53 vs 0.71).

0.17 units/ml) was slightly greater than that of 7 rabbits (0.71 ± 0.14 units/ml) pretreated with atropine prior to exposure to hypoxia. However, this moderate antagonistic effect of atropine on ESF production was not significant.

Rabbits pretreated with atropine and exposed to hypoxia for 18 hr (Table II)

TABLE II. Plasma ESF Levels in Rabbits Exposed to Hypoxia for 18 hr.[a]

| Control | | Atropine pretreated | |
|---|---|---|---|
| Expt. no. | ESF (units/ml) | Expt. no. | ESF (units/ml) |
| 1 | 3.84 | 1 | 1.60 |
| 2 | 6.44 | 2 | 1.48 |
| 3 | 2.60 | 3 | 1.36 |
| 4 | 4.40 | 4 | 0.80 |
| 5 | 6.40 | 5 | 0.80 |
| 6 | 6.00 | 6 | 0.40 |
| | 4.95 ± 0.65 | | 1.09 ± 0.2 |

[a] ± = standard error of mean; $0.001 < p < 0.01$ (4.95 vs 1.09).

showed significantly ($p < .01$) less ESF in plasma than that of untreated control rabbits subjected to the same hypoxic stimulus.

It is quite possible that the antagonism of atropine on ESF production was due to a possible ganglionic blocking action rather than its antimuscarinic effects. Therefore, studies were carried out in rabbits to determine whether the dosage of atropine (5 mg/kg) used to inhibit ESF production was sufficient to antagonize the ganglionic stimulating properties of DMPP on femoral arterial blood pressure. The response to 20 μg/kg of DMPP on blood pressure was the same before and after 5 mg/kg of atropine iv or ip. Thus, the antagonistic effect of atropine on ESF production would not appear to be due to ganglionic blockade.

Studies were carried out in polycythemic mice injected with ESF alone (0.2 units/mouse), ESF plus atropine (5 mg/kg/mouse) and atropine alone, in order to determine whether atropine interfered with the action of ESF on erythroid cells or else produced a decrease in the utilization of ESF.

The polycythemic mouse is known to be resistant to erythropoietic stimuli which enhance endogenous production of ESF. Therefore, if atropine was found to antagonize exogenous ESF in polycythemic mice it might be best explained by decreased utilization or a direct inhibition of ESF at the level of the bone marrow. As shown in Table III, no

TABLE III. Effects of Erythropoietin and Atropine on Radioactive Iron Incorporation in RBC of Polycythemic Mice.[a]

| Treatment | Iron 59 incorporation in RBC |
|---|---|
| Saline | 0.902 ± 0.23(15)[b] |
| Erythropoietin, 0.2 units/mouse | 13.970 ± 4.5 (15) |
| Erythropoietin, 0.2 units/mouse + atropine (5 mg/kg/mouse) | 13.702 ± 3.9 (20) |
| Atropine (5 mg/kg/mouse) alone | 1.391 ± 0.2 (15) |

[a] ± = standard error of mean.

[b] Number of mice is given in parentheses.

significant difference in $^{59}Fe$ incorporation in RBC was seen in polycythemic mice injected with ESF alone or ESF plus atropine. Therefore, the most plausible explanation for the inhibitory effects of atropine is decreased production of erythropoietin.

*Discussion.* In the present studies, rabbits pretreated with atropine were found to produce significantly less ESF in response to hypoxia than that of untreated rabbits exposed to hypoxia (8). Atropine also failed to modify the effects of exogenous ESF on $^{59}FE$ incorporation in RBC in polycythemic mice.

Therefore, it is postulated from these data that atropine inhibits production of ESF rather than interfering with the action of ESF directly on the bone marrow.

In considering possible mechanisms by which atropine might inhibit ESF production, it is of interest to review several recent reports on cholinergic mechanisms involved in the control of the chemoreceptor system, as well as the microcirculation. Latner (9, 10) reported that after destruction of the carotid and aortic bodies, rabbits showed an initial anemia and reticulocytosis and later normalization of the blood picture. On the other hand, Grant (11, 12) demonstrated a marked polycythemia in rabbits after destruction of the carotid bodies and exposure to 150 hr hypoxia. Baciu (13, 14), with the use of cross circulation experiments, reported that cerebral hypoxia stimulated the release of a hypothalamic erythropoietic stimulating factor.

Recently, Tramezzani *et al.* (15) reported that the carotid body produces an ESF-like substance. Cats failed to increase their reticulocytes in response to chronic bleeding after removal of the carotid bodies. Injections of extracts from the carotid bodies of cats exposed to a bleeding stimulus produced an increase in reticulocytes in the blood of normal cats, as well as cats with carotid bodies removed. It was postulated from these studies that the carotid bodies were a source of extrarenal ESF.

Specialized cells localized in the posterior hypothalamus have been postulated to liberate (16, 17) a factor which acts on the kidney to release ESF or else activates the renal erythropoietic factor (REF). It is quite possible that atropine, in the dosage employed in our studies, could inhibit afferent cholinergic transmission from the carotid body along the Hering-Castro nerve resulting in failure of the rabbit to adapt by increasing ESF production in response to hypoxia. Transmission of nerve impulses from the carotid body has been reported to be blocked by appropriate doses of anticholinergic agents (18, 19). It is also possible that atropine could block the effects of the hypoxic stimulus at a site within the kidney.

Finally, McCuskey (20) reported dilatation of the postcapillary sphincter and liberation of reticulocytes in the microcirculation of the fetal rabbit liver following administration of ESF. This investigator[3] also visualized early denucleation of normoblasts in the bone marrow by local application of ESF. Altura (21), using the rat mesocecum, observed that atropine, when applied locally, led to a transient constriction of arterioles, metarterioles. Atropine also antagonized the vasodilatation induced in the microcirculation by the topical application of acetylcholine. It is quite possible that atropine could also block the vasodilatation in the kidney produced by hypoxia which may be an essential part of a release mechanism for ESF following hypoxia.

*Summary.* Atropine was found to inhibit the elevation in plasma ESF levels in rabbits following exposure to hypoxia. The effects of ESF on radioactive iron incorporation in red cells of polycythemic mice was not modified by atropine. The dosage of atropine used in these studies was sufficient to antagonize the muscarinic effects of acetylcholine but not the ganglionic stimulating properties of DMPP on arterial blood pressure in rabbits. The present studies suggest a cholinergic mechanism in the erythropoietic response to hypoxia.

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