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ERYTHROPOIETIN PRODUCTION IN HYPOXIC-HYPERCAPNIC RATS

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DISCLAIMER

The stimulation of erythropoiesis by hypoxia in mice[1] and young rats[2] is severely suppressed when the CO_2 of the inspired air is increased to 5 per cent or 10 per cent. Plethoric mice breathing 5 per cent CO_2 respond to exogenous erythropoietin, but the production of endogenous erythropoietin hypoxic normal mice breathing 5 per cent is markedly reduced[1].

Male Sprague-Dawley rats weighing about 350 gm were exposed in a lucite chamber to gas mixtures containing 10 per cent O_2 and 90 per cent N_2, 10 per cent O_2 and 10 per cent CO_2 with 80 per cent N_2, or 20 per cent O_2 and 10 per cent CO_2 with 70 per cent N_2 which flowed through the chamber at a rate of 3 l/min. After periods of exposure, rats were removed from the chamber, anesthetized with ether, and bled from the dorsal aorta. One ml of rat serum was injected subcutaneously for erythropoietin determinations using a modification[3] of the post-CO plethoric mouse assay initially described by Fogh[4]. Units of erythropoietin were determined from a dose-response curve prepared using the International Reference Preparation human urinary erythropoietin. The results are shown in Table 1. It is evident that a significant reduction in serum erythropoietin levels occurs in hypoxic rats breathing 10 per cent CO_2 after only 4 h. Erythropoietin could not be detected in serum from rats exposed 16 h to the same gas mixture. If hypercapnia was established by a 16-h exposure to 20 per cent O_2 and 10 per cent CO_2 prior to a 4-h exposure to a 10 per cent O_2 and 10 per cent CO_2 gas mixture, the erythropoietic activity in the rat's serum fell to the just detectable level of 1.9 ± 0.09 per cent 72-h ^{59}Fe incorporation rather than the 8.2 ± 0.94 per cent value observed in rats exposed to hypercapnia and hypoxia simultaneously.

Gurney and co-workers[5] have shown that erythropoiesis can be reinitiated in a plethoric mouse by a brief hypoxic exposure. This erythropoietic response is completely abolished by injection of anti-erythropoietin

antibody[3], suggesting that erythropoietin is either released or more likely produced as a result of the hypoxic exposure. If erythropoietin is produced, the system provides a useful model for studying the biogenesis of erythropoietin; indeed, Whitcomb and Moore[6], using this system, have presented evidence for an inhibitor of erythropoiesis in the plasma of plethoric animals. The 72-h ^{59}Fe incorporation into red blood cells of plethoric mice (average hematocrit of 60 per cent at the time of sacrifice) was significantly ($P < 0.001$) increased from 0.57 ± 0.05 per cent to 12.6 ± 0.94 per cent by a 6-h exposure to hypoxia (22,000 ft.). If the plethoric mice were injected intraperitoneally with 1 ml. of serum obtained from rats exposed to a gas mixture of 10 per cent O_2 and 10 per cent CO_2 for 20 h, the 72-h ^{59}Fe incorporation significantly ($P < 0.001$) increased from 12.6 ± 0.94 per cent to 23.8 ± 0.73 per cent. Injection of serum taken from rats similarly exposed to 20 per cent O_2 and 10 per cent CO_2 also significantly ($P > .01$) increased the ^{59}Fe uptake of hypoxic plethoric mice but to only 16.6 ± 0.92 per cent. The potentiation of the erythropoietic response in plethoric mice was much greater using the hypercapnic-hypoxic sera than the hypercapnic sera ($P < 0.001$). Erythropoietin was not detectable in either serum sample. Thus, sera from hypercapnic rats potentiates rather than inhibits the biogenesis of erythropoietin. The nature of the substance responsible for this potentiation is unknown, although some preliminary evidence suggests it is a protein.

The mechanism responsible for the suppression of erythropoietin production by hypercapnia is unknown. Faura and co-workers[1] suggest that the induced hyperventilation increases the hemoglobin oxygen saturation, and the delivery of O_2 to the tissues is increased so that the effect of hypoxia is overcome. We feel that the alterations in acid-base balance induced by

hypercapnia are also important. The data presented here suggest that the hypercapnic rat does detect hypoxia with the production of some substance involved in the biogenesis of erythropoietin, but this substance cannot be converted into the active hormone in the hypercapnic animal. Measurements of the acid-base changes and of arterial oxygen saturation in the hypercapnic-hypoxic state may clarify this problem.

REFERENCES

1. Faura, J., Gurney, C. W., and Fried, W., Ann. N. Y. Acad. Sci. 149: 456 (1968).
2. Pepelko, W. E. J., Appl. Physiol. 28: 646 (1970).
3. Schooley, J. C., and Mahlmann, L. J., Submitted to Proc. Soc. Exp. Biol. Med. 1971.
4. Fogh, J., Scandinav. J. Clin. Lab. Invest. 18: 33 (1966).
5. Gurney, C. W., Munt, P., Brazell, I., and Hofstra, D., Acta haemat. 33: 246 (1965).
6. Whitcomb, W. H., and Moore, M. Z., J. Lab. Clin. Med. 66: 641 (1965).

Table 1

Erythropoietic activity of plethoric LAF_1 mice injected with serum taken from male rats exposed to hypoxia and hypoxia with hyperkapnia

Duration of exposure	Treatment	72-hr. ^{59}Fe incorporation	I.R.P. units EPO/ml serum
4 hours	10% O_2	17 ± 1.0*	1.0
	10% O_2 and 10% CO_2	8.2 ± 0.94	0.25
8 hours	10% O_2	32 ± 0.90	> 2.9
	10% O_2 and 10% CO_2	1.7 ± 0.30	Detectable
16 hours	10% O_2	18 ± 1.3	1.0
	10% O_2 and 10% CO_2	0.77 ± 0.07	NS
	Saline	0.56 ± 0.04	-

* Standard error of the mean. 6-8 mice/group.