Is the Intracellular Calcium-Mediated Pathway Involved in Erythropoietin-Induced Hypertension?

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phore, ionomycin (Wako Pure Pharmaceutical, Tokyo, Japan), with a chemotactic peptide, FMLP (Peptide Institute, Osaka, Japan), or with epoetin- α (EPO, Kirin Beer Co. Ltd., Tokyo, Japan). In a short coculture study, at a concentration of 10,000 mlU/ml of rhEPO, morphological changes in ECs and SMCs

Dear Sir,

Hypertension is one of the adverse effects associated with recombinant human erythropoietin (rhEPO) therapy for anemia in hemodialysis patients. The incidence of hypertension is reported to be 10-15% [1,2], sometimes requiring a reduction in dosage or discontinuation of rhEPO therapy [2]. The exact mechanism of rhEPO-induced hypertension has not yet been fully elucidated, although several theories have been advanced. Of these, an attractive possibility is a direct vasopressor effect of rhEPO as suggested by two recent reports [3, 4]. We examined whether rhEPO directly affects the major components of the vessel wall, the endothelial cells (ECs) and smooth muscle cells (SMCs), through the intracellular calcium-mediated pathway which plays an important role in vascular contraction induced by angiotensin II and vaso-pressin [5].

ECs were prepared from an endothelial cell line derived from the human umbilical cord (Endocell, Kurabo Industry Ltd., Osaka, Japan). SMCs were from a smooth muscle cell line of the rat thoracic aorta, A10 (Dainippon Pharmaceutical Co. Ltd., Osaka, Japan). Both cell types were cultured with Dulbecco's minimum essential medium (Flow Laboratories Inc., Va., USA) for 7 days. The confluent cells were trypsinized to disperse, and then loaded with 2 μ M fura 2-AM (Dojin Chemical, Kumamoto, Japan) for 15 min at 37°C and resuspended at 1×10^6 in a HEPES buffer. Intracellular free calcium ($[Ca^{2+}]_i$) levels were determined according to the method of Grynkiewicz et al. [6], using a two-wave spectrofluorophotometer, RF-5000 (Shimadzu Co. Ltd., Kyoto, Japan). Changes in the $[Ca^{2+}]_i$ levels of these cells were examined by stimulation with a calcium iono-

$[Ca^{2+}]_i$ nM 300 –

ionomycin (50nM)

250 –

200 –

$\Delta F/F$

10 min

EPO (70,000mU/ml)
 150 ■ 100-50-150 100
 50 150
 100
 50
 10 min

Fig. 1. Changes in $[Ca^{2+}]_i$ of endothelial cells stimulated by EPO and ionomycin. were observed for 30 min using phase-contrast microscopy.

As shown in figure 1, the baseline $[Ca^{2+}]_i$ level of the ECs was 95-110 nM. Following stimulation by ionomycin (50 nM), $[Ca^{2+}]_i$ increased to a peak of 280 nM at a time of about 30 s. Following stimulation with EPO

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at different concentrations ranging from 7.0-70,000 mIU/ml, no significant changes in $[Ca^{2+}]_i$ were noted during the observation period of 10 min. Also, no significant increase in $[Ca^{2+}]_i$ was observed by FMLP at 100 nM. Although not shown, changes in the $[Ca^{2+}]_i$ levels of SMCs by ionomycin-stimulation were noted as in the case of ECs, but such changes were absent with FMLP and EPO. No morphological changes of ECs or SMCs were observed following the addition of EPO (10,000 mIU/ml).

As a cause of rhEPO-induced hypertension, Heidenreich et al. [4] suggested a direct vasopressor effect of rhEPO based on the results showing that isolated rat renal resistance vessels contract without time delay with the addition of rhEPO at concentrations ranging from 10,000 to 20,000 mIU/ml. They also described that this contractile response was not abrogated after the enzymatic removal of the ECs, but disappeared in the absence of extracellular Ca^{2+} , suggesting involvement of the intracellular Ca -mediated pathway of the vascular SMCs. Tepel et al. [7] reported that changes in the $[Ca^{2+}]_i$ levels of platelets by rhEPO were significantly greater in genetically hypertensive rats than in normotensive Wistar Kyoto rats, suggesting that genetic differences influence the responsiveness to rhEPO. An increase in the $[Ca^{2+}]_i$ level of the erythroid precursor cells was observed after stimulation by rhEPO [8], in which response the receptor for rhEPO probably played a role. Although high- and/or low-affinity receptors for rhEPO have been demonstrated on the surface of erythroids and megakaryocytes [9], only one report has described low-affinity receptors in ECs [10], and none has described them in SMCs. The fact that the receptor for FMLP is limited to phagocytic cells, may account for the lack of response to EPO in our own experiment.

In contrast to the results of Heidenreich et al. [4] and Tepel et al. [7], the present results seem to be consistent with those of Pagel et al. [11] and Bund et al. [12], both of which dispute a direct vasopressor effect of erythropoietin. At least two possibilities may explain this disagreement regarding the vascular effect of rhEPO. First, the rhEPO dosages used by Heidenreich et al. [4] and Tepel et al. [7] were much greater (10,000-250,000 mIU/ml) than our experimental dosage and physiological levels. Physiological plasma erythropoietin levels range from 10 to 20 mIU/ml [9]. Even when the plasma rhEPO levels reached a peak value of about 1,000-2,300 mIU/ml just after the intravenous injection of 3,000-6,000 U rhEPO, no immediate rise in blood pressures was

reported. The plasma level of injected rhEPO rapidly reaches an undetectable level within 48 h with a half-life of 5-7 h in hemodialysis patients [13]. There seems to be a great gap between the plasma rhEPO levels in hemodialysis patients and in those in vitro experiments affirming a vasopressor effect of rhEPO. Differences in platelet $[Ca^{2+}]_i$ levels were not reported to correlate with those in mean blood pressure of dialysis patients [14]. Second, according to Tepel et al. [7], genetic differences and/or the hypertensive state may influence $[Ca^{2+}]_i$ responsiveness of platelets to rhEPO, with this point requiring further study. Although a special mechanism limited to the uremic environment cannot be ruled out, we were not able to find an in vitro direct effect of EPO on $[Ca^{2+}]_i$ levels of the vascular cell lines.

The calcium-mediated pathway of ECs and SMCs seems unlikely to be associated with rhEPO-induced hypertension.

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