expression of the cyclohydrase. Since NO-mediated hypotensive crises are not likely to last more than a few days, physiologically significant depletion of the BH₄ pool supporting neurotransmitter synthesis is unlikely.

3) In endothelial cell studies by Schmidt et al. (6), loss of the constitutive NOS activity following BH₄ depletion was seen when calcium ionophores were used to elicit maximum NO- synthesis, but basal NOS-synthesis was not affected. The implications of this finding are unclear, since it is not known if the physiological rate of NO- production by vascular endothelium more closely approximates the basal or the maximally stimulated rate. The finding that agents stimulating constitutive NOS cause hypotension in normal animals indicates, however, that the enzyme is not fully activated in vivo (7).

4) Although aggressive BH₄ depletion apparently inhibits constitutive NOS activity in cultured cells (6), it is noted that this isoform is presumably saturated with BH₄ in vivo and that most studies show the cofactor to be tightly bound. On the other hand, cytokine-stimulated expression of the inducible NOS apoenzyme does not result in NOS activity unless stoichiometric amounts of BH₄ are available. Depletion of BH₄ is thus expected to limit inducible NOS activity more directly and dramatically than the constitutive isoform.

The foregoing discussion not withstanding, the comments and extensive in vitro studies by Wachter and co-workers usefully focus our attention on issues which must be addressed before BH₄ synthesis inhibitors can be confidently and rationally used therapeutically in NO-mediated shock.

H. Wachter and co-workers raise several concerns regarding our suggestion that blocking tetrahydrobiopterin (BH₄) synthesis may selectively limit nitric oxide (NO) overproduction due to inducible nitric oxide synthase (NOS) (7). Although such questions can be definitively answered only by in vivo studies, we remain optimistic with respect to the therapeutic approach for the following reasons:

1) In vitro studies in cytokine-stimulated endothelial cells show almost complete inhibition of NO-formation by the guanosine triphosphate cyclohydrase inhibitor 2,4-diamino-6-hydroxyprpyridine (DAHP) (2). Endothelial cells are more likely than fibroblasts to be a physiologically important source of NO because of their proximity to the vascular smooth muscle.

2) DAHP depletes plasma and liver BH₄ by 90% or more but causes only modest depletion of brain BH₄ (3,4). Such inhibition had little effect on neurotransmitter levels, and few or no signs of neurotransmitter deficiencies were reported in those studies (3,4) or in other studies (5) of mice bearing a mutation resulting in a defective

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