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LETTERS TO THE EDITOR

Glutamine-mediated nitric oxide synthase inhibition might explain the 'arginine paradox'

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From several studies, it has been described that nitric oxide synthase(NOS) activity increases if extracellular arginine levels are elevated [1,2] despite of the high intracellular amino acid level (0.1 - 2 mM in endothelial cells [3]), which would maintain the enzyme saturated [4]. This 'arginine paradox' may be explained by several mechanisms including amino acid transport, translational control and the presence of endogenous NOS inhibitors [5,6].

Increasing extracellular arginine concentration (10-1000 μM), in a range including levels found in neurologic patients [7], favors an increased cytokine-activated inducible NOS (iNOS) activity in cultured astrocytes through a translational mechanism. Also, at the 100-μM extracellular level, arginine increases nitrite synthesis by endothelial cells [8].

However, other studies show NOS activity may be independent of extracellular arginine levels. For example, arginine administration does not cause vasorelaxation or alter blood pressure according to some studies [9,10]. Also, astrocyte nitric oxide (NO) synthesis is reduced even in the presence of increased extracellular arginine levels when argininosuccinate synthetase (AS, the rate limiting enzyme in arginine synthesis) is inhibited.

Moreover, AS activity is inhibited by NO, so possibly an increased NOS activity uncouples arginine levels and NO synthesis. Furthermore, arginine and citrulline are competitive inhibitors of dimethylarginine dimethylaminohydrolase (which catalyzes hydrolysis of endogenous NOS inhibitors) [11]; this may increase the levels of methylarginines, thus inhibiting NOS independently of arginine levels [12].

Some studies [7] are consistent with those previous reports since no correlation between arginine and NOx was reported, suggesting that, at least as shown by cerebrospinal fluid (CSF) concentrations, NO synthesis in the central nervous system (CNS) is independent of arginine levels.

In fact, increasing extracellular arginine concentrations increases NO synthesis in arginine-depleted cells only [13]. This evidence suggests the dependence of NOS activity on arginine levels is related to an additional mechanism.

According to some studies [7], glutamine modulates NO synthesis. Increasing extracellular arginine concentration (up to 10 mM) in the absence of glutamine in a culture media does not alter bradykinin-induced NO synthesis but increases it when glutamine is also present [14].

Furthermore, high extracellular glutamine concentrations inhibit iNOS activity [15]. Glutamine (600 μM) also inhibits endothelial NOS activity. This inhibition is reverted upon the addition of arginine (10 μM and above) just as the 'arginine paradox' predicts.

Glutamine inhibits bradykinin-induced NO synthesis if intracellular arginine concentration is below 3 mM, only higher arginine concentrations avoid glutamine inhibition. This regulation is likely to occur *in vivo* since, in endothelial cells, intracellular glutamine concentration may be more than 20-fold higher than arginine, and it is reduced up to 98% during NO synthesis allowing enzyme activity.

Furthermore, it may occur in the human CNS since mean CSF glutamine and arginine concentrations in neurologic patients were 505 (143-1830) μM and 19 (0.1-99) μM, respectively [7]. Actually, this solution to the 'arginine paradox' was proposed 20 years ago by *in vitro* studies and is now supported *in vivo* [7]. The 'arginine paradox' was previously solved for iNOS through an arginine-modulated translational mechanism; our proposed mechanism is likely to be related to the constitutive isoforms since it is independent of infection and inflammation[7]. Experimentally, NOS activity may be modulated by manipulating arginine levels[1, 2]; physiologically, this seems to be modulated by glutamine concentration[7].

Modulation of these mechanisms is important since NOS inhibition is considered a possible therapeutic strategy for some disorders involving oxidative stress, not only in the nervous system but also cardiovascular disease[16], and Coronavirus disease-19[17] among many others. Both arginine[17] and glutamine[18] supplementation have been tested to modulate NO synthesis in humans, so their reciprocal modulation should be taken into account.

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