Potent and Selective Inhibition of Neuronal Nitric Oxide Synthase by N^{ω}-Propyl-L-arginine

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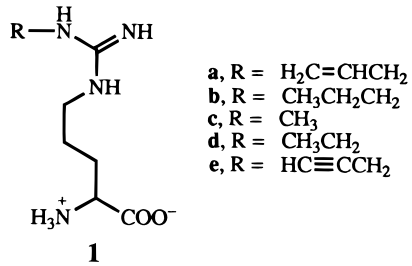
Nitric oxide, now known to function as a hormone and neurotransmitter in a variety of physiological processes, is biosynthesized from L-arginine by a family of isoforms collectively known as nitric oxide synthase (NOS, EC 1.14.13.39).[1] The three major enzyme isoforms include the endothelial cell enzyme (eNOS), which is involved in the regulation of smooth muscle relaxation and blood pressure, neuronal nitric oxide synthase (nNOS), important to brain development and memory, and an inducible form (iNOS) produced by activated macrophage cells during an immune response. The endothelial and neuronal isoforms are constitutive and calmodulin-dependent, whereas the macrophage enzyme is inducible and calmodulin−independent because it contains tightly bound calmodulin. All of the isoforms have a multicofactor requirement in two binding domains: the N-terminal domain contains the oxygenase activity and binds heme and tetrahydrobiopterin (and the substrate) and the C-terminal domain, which has the reductase activity, binds the NADPH, FAD, and FMN cofactors. The calcium-dependent regulatory protein calmodulin binds between the cofactor domains and may be involved in electron transfer between the domains.[2]

Excess production of nitric oxide by the NOS isoforms has been implicated in a variety of diseases,[3] such as stroke, Alzheimer's disease, and other neurodegenerative diseases,[4] septic shock,[5] inflammatory arthritis,[6] and colitis.[7] When this occurs, inhibitors of NOS would be an important approach to decrease the concentration of nitric oxide in the cell. However, because of the importance of nitric oxide to physiological functioning, it is essential that potent and selective inhibitors of the isoforms be developed. Many different inhibitors of NOS are known; some of the earliest inhibitors include the N^{ω}-substituted-L-arginine analogues.[8,9] Recently, we reported the inactivation of nNOS by N^{ω}-allyl-L-arginine (**1a**) and showed that N^{ω}-propyl-*L*-arginine (**1b**) also was a competitive inhibitor and inactivator of nNOS.[10] Here we report the unexpected finding that the inhibition of nitric oxide synthases by N^{ω}-propyl-L-arginine is highly selective for nNOS.

Table 1. Comparison of K_i (or IC_{50}) Data for N^{w}-Substituted-L-arginine Analogues

	K_i (nM)			selectivity[a]	
1 (R)	nNOS[b]	iNOS[c]	eNOS[d]	nNOS/iNOS	nNOS/eNOS
propyl	57	1.8×10^5	8500	3158	149
allyl	200	2100	3100	10.5	15.5
propargyl	430	620	810	1.4	1.9
methyl[e]	10000[f]	14000[f]	5900[f]	1.4	0.6
ethyl[e]	16000[f]	6100[f]	9500[f]	0.4	0.6

[a] Selectivity for nNOS/iNOS is the ratio of the inverse of the K_i or IC_{50} values, since the lower the K_i or IC_{50}, the more potent the inhibition. [b] Purified as described in Zhang et al.[10] [c] Purified as described in Hevel et al.[12] [d] Purified as described in Martasek et al.[13] [e] Data taken from Moore et al.[9] [f] IC_{50} values, not K_i values.



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N^{ω}-Propyl-L-arginine[10] was found to be a competitive inhibitor[11] of all three isoforms. On the basis of the K_i values with each isoform (Table 1), it is apparent that there is a considerable degree of selectivity in favor of nNOS. The potency of inhibition of nNOS (from bovine brain)[10] by N^{ω}-propyl-L-arginine is 3158 times that of iNOS (mouse murine recombinant)[12] and 149-fold that of eNOS (bovine endothelial recombinant).[13] To the best of our knowledge, this nNOS/iNOS selectivity is one of the largest, if not the largest, degrees of selectivity reported; the selectivity of nNOS over eNOS also is fairly substantial. This is quite unexpected, given that the selectivity factors for N^{ω}-methyl-L-arginine (**1c**) and N^{ω}-ethyl-L-arginine (**1d**) are only about 2;[9] interestingly, N^{ω}-methyl-L-arginine is slightly selective for nNOS and eNOS over iNOS, whereas N^{ω}-ethyl-L-arginine is slightly selective for iNOS over nNOS and eNOS. Furthermore, we have found that putting unsaturation into the propyl side chain has a dramatic, undesirable effect on selectivity. Both N^{ω}-allyl-L-arginine and N^{ω}-propargyl-L-arginine (**1e**) are weakly selective, the former having a selectivity of nNOS/iNOS of about 10 and nNOS/eNOS of 15, whereas the latter has a selectivity of less than 2. It is apparent that the geometry and size of the side chain are extremely important to the selectivity of inhibition.

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[||] Provided the recombinant *E. coli* cells expressed with murine macrophage iNOS.
[⊥] Provided the recombinant eNOS.

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(11) The competitive reversible inhibition of the isoforms by N^{ω}-propyl-*L*-arginine was studied under initial rate conditions with the hemoglobin assay as described in ref 10. The concentrations of L−arginine used were 3, 4, 6, 10, and 15 μM for nNOS; 10, 20, 30, and 60 μM for iNOS; and 2, 4, 8, and 15 μM for eNOS. The concentrations of N^{ω}-propyl-L-arginine used were 0.05, 0.1, 0.15, and 0.2 μM for nNOS; 50, 100, 150, and 200 μM for iNOS; and 10, 20, 30, and 40 μM for eNOS. Data were analyzed by the methods of Dixon (Dixon, M. The determination of enzyme inhibitor constants. *Biochem. J.* **1953**, *55*, 170−171) and Cornish-Bowden (Cornish-Bowden, A. *Fundamentals of Enzyme Kinetics*; Portland Press: London, 1995; pp 102−108).

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