EVIDENCE FOR A BIFUNCTIONAL ROLE OF NITRIC OXIDE IN THE REGULATION OF HUMAN ASTROCYTOMA U-373 MG CELL PROLIFERATION

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NO plays remarkably diverse functions in key physiological processes such as smooth muscle relaxation, immune cell-mediated microbial killing and platelet aggregation inhibition (Moncada et al., 1991). Within the central nervous system, NO is a crucial component of the signal transduction pathways used for memory formation, sensory processing and cerebral blood flow regulation. However, under excessive production, NO can initiate a cascade of neurotoxic reactions which culminate in cell death. One of the most toxic interactions of NO has been suggested to be with DNA and DNA repairing enzymes such as the ribonuclease reductase which leads to DNA damage, inhibition of cell division and/or death (Moncada et al., 1991).
An intriguing aspect of the biology of NO is its strict relationship with intracellular calcium ion concentration ($[Ca^{2+}]_i$) which comprises physiological NO regulation of intracellular Ca^{2+} homeostasis in many cell types, including neurons (Clementi E., 1998). Earlier observations in our laboratory established a role for the NO/Ca^{2+} signaling in the pyrogenic effect of IL-1β (Palmi et al., 1992; Palmi et al., 1994; Palmi et al., 1996) and showed with two different experimental models of human astrocytoma cells and rat brain slices, that NO production in response to IL-1β or its release from NO donors such as diethylamine/NO and spermine/NO complexes were responsible for intracellular Ca^{2+} mobilisation from intracellular Ca^{2+} pools (Meini A. et al., 2000; Palmi and Meini, 2002).
Studies with the these compounds revealed a dual role of NO in the Ca^{2+} response which depended on the amount of NO released. Low NO levels therefore stimulated while high NO concentrations inhibited Ca^{2+} release.
Since depending on its intracellular level, Ca^{2+} is an important regulator of either cell growth and cell death or differentiation (Berridge M.J., 1998), we prompted a study to investigate the role of NO/ Ca^{2+} signaling on cell proliferation.
To do this changes in $[Ca^{2+}]_i$ were correlated with those in proliferation of a human U-373 MG astrocytoma cell line treated with different (1, 10, 300, 1000, 10000 μM) concentrations of (DETA/NO) [(z)-1-[2-(2-Aminoethyl)-N-(2-ammonio-ethyl)amino]diazen-1-ium-1,2-diolate]].
Measurement of $[Ca^{2+}]_i$ and cell proliferation. Cells at a density of $5x10^5$ per ml were plated on Petri dishes containing circular glass coverslips. Following confluence the cells were treated 1h with DETA/NO and then reincubated at 37°C for 24, 48, 72 hr in 95% air + 5% CO_2 . Thereafter the glass carrying adhering cells were removed from the Petri dishes, loaded with the Ca^{2+}-sensitive dye, fura-2, for 30 minutes and the fluorescence determined at 340 nm excitation and 505 nm emission wavelenghts. Cell Proliferation was determined at 24, 48, 72 hr by using either the immunofluorescence antibodies to BrdU after BrdU incorporation or the fluorochrome proliferative reagent Hoechst 33342.
Our results showed a concentration-dependent effect of NO on cell proliferation which depended on differential Ca^{2+} signaling. Thus low (1-10 μM) DETA/NO levels increased $[Ca^{2+}]$i and stimulated cell proliferation while high DETA/NO levels (0.3-10 mM) failed to elicit this effect and resulted in progressive inhibition of cell growth. Interesting, DETA/NO-induced elevation in $[Ca^{2+}]_i$ and cell proliferation was potentiated by the presence of bepridil (10 μM), a known inhibitor of the Na^+- Ca^{2+} exchanger.
Since astrocytes have been recently shown to stimulate neurogenesis (Song H. et al., 2002), our results raise the possibility that modulation of NOS activity might be a potential therapeutic target in a wide range of brain disorder characterised by a progressive and extensive neuronal cell degeneration.

References

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