Endothelin-1 is over-expressed in amyotrophic lateral sclerosis and induces motor neuron cell death

Eugenia Ranno , Simona D'Antoni , Michela Spatuzza , Antonio Berretta , Floriana Laureanti , Carmela M. Bonaccorso , Rosalia Pellitteri , Patrizia Longone , Alida Spalloni , Anand M. Iyer , Eleonora Aronica , Maria Vincenza Catania

A B S T R A C T

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disorder characterized by progressive loss of motor neurons (MNs) and astrogliosis. Recent evidence suggests that factors secreted by activated astrocytes might contribute to degeneration of MNs. We focused on endothelin-1 (ET-1), a peptide which is strongly up-regulated in reactive astrocytes under different pathological conditions. We show that ET-1 is abundantly expressed by reactive astrocytes in the spinal cord of the SOD1-G93A mouse model and sporadic ALS patients. To test if ET-1 might play a role in degeneration of MNs, we investigated its effect on MN survival in an *in vitro* model of mixed rat spinal cord cultures (MSCs) enriched of astrocytes exhibiting a reactive phenotype. ET-1 exerted a toxic effect on MNs in a time- and concentration-dependent manner, with an exposure to 100–200 nM ET-1 for 48 h resulting in 40–50% MN cell death. Importantly, ET-1 did not induce MN degeneration when administered on cultures treated with AraC (5 μM) or grown in a serum-free medium that did not favor astrocyte proliferation and reactivity. We found that both ET_A and ET_B receptors are enriched in astrocytes in MSCs. The ET-1 toxic effect was mimicked by ET-3 (100 nM) and sarafotoxin S6c (10 nM), two selective agonists of endothelin-B receptors, and was not additive with that of ET-3 suggesting the involvement of ET_B receptors. Surprisingly, however, the ET-1 effect persisted in the presence of the ET_B receptor antagonist BQ-788 (200 nM–2 μM) and was slightly reversed by the ET_A receptor antagonist BQ-123 (2 μM), suggesting an atypical pharmacological profile of the astrocytic receptors responsible for ET-1 toxicity. The ET-1 effect was not undone by the ionotropic glutamate receptor AMPA antagonist GYKI 52466 (20 μM), indicating that it is not caused by an increased glutamate release. Conversely, a 48-hour ET-1 treatment increased MN cell death induced by acute exposure to AMPA (50 μM), which is indicative of two distinct pathways leading to neuronal death.

Altogether these results indicate that ET-1 exerts a toxic effect on cultured MNs through mechanisms mediated by reactive astrocytes and suggest that ET-1 may contribute to MN degeneration in ALS. Thus, a treatment aimed at lowering ET-1 levels or antagonizing its effect might be envisaged as a potential therapeutic strategy to slow down MN degeneration in this devastating disease.