Differing effects of polyamines on nitric oxide synthase

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In the central and peripheral nervous system L-arginine acts as a common substrate for a number of metabolic reactions including the production of polyamines and nitric oxide (NO). NO has become established as a diffusible messenger mediating cell-cell interactions including neuronal signalling [1]. Recent research has shown that an excess or shortage in NO production has been linked with various disease states.

Nitric oxide synthase (NOS) which produces NO is modulated by several compounds including polyamines [2]. Polyamines are widely present in nervous tissue and are known to play a role in cell division and other cell functions. Polyamines are thought to be capable of modulating NMDA receptor function via a polyamine site present outside the cell membrane which in turn can have an effect on NOS activity by regulating calcium entry. On the other hand polyamines may have a direct effect on controlling the NOS activity in the brain. We have tested the effects of putrescine, spermidine and spermine which could be produced from the same substrate L-arginine used to synthesize NO. These amines could act as a novel modulator of NOS in vivo in brain. The activation and/or inhibition pattern of putrescine, spermidine and spermine was studied on rat cerebellar and cortex NOS in vitro.

Rat cerebellar and cortex tissue were isolated and prepared for NOS activity [2] in the presence and absence of polyamines at different concentrations. NOS was extracted by sonication using buffer containing 0.25M sucrose, 100 mM Tris, pH 7.4. Extraction buffer also contained 1 mM dithiothreitol, 1 mM EDTA, 100 μg/ml PMSF, 10 μg/ml leupeptin, 10 μg/ml soyabean trypsin inhibitor, 2 μg/ml aprotinin. NOS activity was measured by following the conversion of L-[U-14C]arginine to citrulline in the presence and absence of the inhibitor L-NG monomethyl arginine. Assays were performed at 37°C in a total of 0.1 ml consisting of 12.5 mM HEPES, pH 7.3 with 1.2 mM MgCl₂, 0.96 mM CaCl₂, 60 mM L-valine, 1.2 mM L-citrulline, 0.024 mM L-arginine, 120,000 dpm radiolabelled L-arginine and 0.12 mM β-NADPH.

Putrescine significantly increased NOS activity which was not dose dependent in cerebellar tissue (Fig.1a). However spermine inhibited NOS activity in dose dependent manner. Spermidine showed no significant inhibition or activation of cerebellar NOS in the concentration range studied. No significant activation or inhibition of rat cortex NOS by all three amines was observed (Fig.1b).

Fig. 1. Effects of putrescine, spermidine and spermine on rat cerebellar (a) and cortical (b) NOS activity. 100% cerebellar NOS activity = 315 ± 79 nmoles of citrulline formed/min/g protein; 100% cortical NOS activity = 86 ± 20 nmoles of citrulline formed/min/g protein. The results are the means ± SEM of 3-4 experiments. * and ** represent p<0.005 and p=0.002 respectively when compared to control activity.

Polyamines are known to be regulators of cell growth and differentiation. They are also thought to be important in cell membrane functions [3]. An immunosuppressive role is postulated in some placental functions [4]. NMDA receptor may have a role in modulating NOS functions which in turn may be modulated by polyamines. Putrescine which is initially produced by ornithine decarboxylase activation may have an activating effect on NOS; however, in the long term when sufficient higher polyamines are accumulated spermine may inhibit NOS activity. This activation and/or inhibition is not so pronounced on the cortex NOS as its in vitro activity is much lower compared to cerebellar NOS.