

P010 The binding of heparin to glial cell line-derived neurotropic factor (GDNF) and the closely related cytokines neurturin and artemin.

Alfano I., Vora P., Mummery R., Mulloy B.* and Rider C.
*School of Biological Sciences, Royal Holloway University of London, Egham Hill, Egham, Surrey TW20 0EX, UK. *National Institute for Biological Standards and Control, Blanche Lane, South Mimms, Potters Bar, Herts, EN6 3QG, UK.*

Glial cell line-derived neurotropic factor, GDNF, is a neuroprotective cytokine with therapeutic potential in Parkinson's disease. In mice, homozygous knock out of the genes encoding GDNF, its specific receptor GFR- α 1, or its signal receptor c-Ret, show selective developmental defects, most strikingly an absence of kidneys. Intriguingly, homozygous disruption of the gene encoding heparan sulphate 2-*O*-sulphotransferase causes a similar phenotype, implying that 2-*O*-sulphate rich heparan sulphate plays an essential role in the developmental activity of GDNF. In support of this we previously showed that rhGDNF binds strongly to heparin, but not selectively to 2-*O*-desulphated heparin. More recently we have found that neurturin and artemin, which are closely related to GDNF, also bind to heparin, in fact with slightly higher affinities than GDNF. However with these two cytokines there is not the same critical role of 2-*O*-sulphates in heparin binding. We have expressed a soluble Flag-tagged construct of the GFR- α 1 extracellular domain and shown that it does not bind to heparin. This construct will be used to study the effect of heparin on GDNF-GFR- α 1 binding. We are also producing recombinant mutants of GDNF with selected substitutions and deletions in order to locate its heparin binding site and determine whether or not this is distinct from the GFR- α 1 binding site.