P-54

Exploring the Effect of Radioligand Depletion on Affinity Determinations in the Dopamine D2 Binding Assay.

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Dopamine D2 receptors are the main target for antipsychotics and they are investigated in many drug discovery programs. One of the standard method to evaluate the affinity of new agents to target receptor is the radioligand binding assay. Recently, the need for a high throughput rate to screen large number of compounds led to miniaturization of assay formats which opened further possibility of process automatization. However, assay miniaturization increases the risk of radioligand depletion – a phenomenon in which the free ligand concentration is significantly reduced which complicates the interpretation of binding data. This problem is particularly acute for dopamine D2 binding assays with the use of high-affinity radioligands, and is often ignored by investigators leading to substantial errors in the obtained values of affinity measurements.
In our studies, we have explored the effects of volume reduction in D2 binding assay from 0.5 ml to 0.25 ml. As a result, troubleshooting procedure applied to correct inaccuracies arising from ligand depletion is presented.

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