Dithiothreitol promotes a higher affinity state of the serotonin transporter for the tricyclic antidepressant, imipramine.

HELOISE M. TARRANT & D. CLIVE WILLIAMS
Department of Biochemistry, Trinity College, Dublin 2, Ireland.

Neurotransmitter transporters are present in nerve terminal membranes and are responsible for the reuptake of released neurotransmitter by resynaptic one. Such a transporter is the serotonin transporter which also represents the site of action of many clinically used tricyclic antidepressants, such as imipramine.

Recently, most of the known Na⁺-dependent neurotransmitter transporters have been cloned and sequenced (for review see (1)). On the basis of sequence similarities, the transporters can be divided into two superfamilies. Within each family many features are conserved which may serve important structural, functional or regulatory roles. One such feature, conserved throughout the superfamily of which the serotonin transporter is a member, is two cysteines, nine amino acids apart, which are located on a putative large extracellular loop of the protein. It has been proposed that these cysteines may be involved in ligand binding and/or substrate uptake by the transporters, or perhaps function in maintaining the transporters in an active conformation (2). Thus, the effects of the sulphydryl reducing agent, dithiothreitol (DTT), on ligand binding to the human platelet serotonin transporter were studied.

When the binding of the tricyclic antidepressant, [3H]imipramine, to human platelet membranes was measured over a range of increasing DTT concentrations, a concentration dependent presence of DTT, relative to the control value determined in the absence of [3H]imipramine concentrations in the presence of DTT. This DTT induced decrease in the KD value was seen to the human platelet serotonin transporter were studied.

The dissociation curve was biphasic in the absence of DTT while in the presence of DTT the curve was either monophasic (slowly dissociating) or tended towards this condition (Fig.1.). The dissociation rate of [3H]imipramine from platelet membranes was measured over a range of DTT concentrations. As the DTT concentrations increased, there was an apparent concentration-dependent shift in the [3H]imipramine dissociation curve from the biphatic condition towards the monophasic state. The effect appeared maximal at 5 mM DTT and no further slowing of the dissociation rate was achieved at higher DTT concentrations.

When dilution-induced dissociation of [3H]imipramine from platelet membranes is carried out in the absence of the transporter substrate, serotonin (0.5 mM), a similar slowing of the [3H]imipramine dissociation rate is observed. This observation is consistent with previously published studies (3) and indicates that both serotonin and DTT induce similar conformational changes in the transporter structure. Further evidence for a DTT-induced conformational change in transporter structure is provided by heat-inactivation studies. Platelet membranes and [3H]imipramine were incubated in the presence and absence of DTT (5mM) at 60°C. Biphatic heat-inactivation curves were obtained in the absence of DTT while curves approaching a monophasic (rapidly inactivated) condition were obtained in the presence of DTT. This indicates that the conformation of the transporter in its thiol-reduced form is more sensitive to heat-denaturation.

The experiments described above were repeated using the non-tricyclic antidepressant, [3H]paroxetine, as a ligand for the serotonin transporter. DTT had no detectable effect on the affinity of [3H]paroxetine for the transporter.

These results demonstrate that the transporter exists in two conformational states in vitro which are determined by thiol oxidation status. This may be crucial in regulation of the structure and function of the transporter by its substrate, serotonin.

H.M.T. acknowledges funding from the Health Research Board.