Ionic requirements of [H]GBR-12935 binding to the dopamine transporter in canine striatal membranes

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The action of the neurotransmitter dopamine, following its synaptic release is terminated by reuptake into the nerve terminals [1]. This process is dependent on sodium and mediated by a membrane located transporter [2]. The transporter has been implicated as a cocaine receptor related to drug self-administration [3].

The dopamine transporter has been studied directly via the accumulation of radiolabelled dopamine by synaptosomes and indirectly via the binding of dopamine transport inhibitors to membrane preparations [4,5]. However, there have been conflicting results reported on the properties of the binding site detected by a number of different dopamine inhibitors. In particular, the ionic dependency and requirement for Na+ varies widely between different inhibitors and different studies. In this study, we have used the potent and selective inhibitor of dopamine transport, GBR-12935 (1-[Z-diphenyl methoxy)ethyl]-4-(3-phenylpropyl)-piperazine) to characterize its binding properties to membrane preparations from canine striatum under a variety of ionic conditions.

Crude synaptosomal membranes from canine striatum were prepared by a modification of the method previously described [6] and resuspended in sucrose (0.32M), Tris, Hepes (5mM) pH 7.4 to yield a final protein concentration of 10-20 mg/ml. Equilibrium binding of [H]GBR-12935 to diluted membranes (final conc 0.08mg protein) was performed by incubating the membranes for 1 h at 22°C in buffer (20mM Hepes/Tris for sodium and chloride substitution experiments and bicarbonate/phosphate buffers for Na+ dependency and saturation analysis). Specific binding was defined as the difference in binding observed in the presence and absence of 3pM mazindol and represented 70-80% of total binding at a 1.0nm concentration of ligand. Binding was terminated by rapid filtration over Whatman GF/B glass filters presoaked in 0.1% bovine serum albumin.

Previous studies have demonstrated that the uptake of [H]dopamine by synaptosomes is dependent on the presence of Na+ and CI [7]. Thus, in the first series of experiments the effect of these ions and others on mazindol-sensitive [H]GBR-12935 binding to canine striatal membranes was investigated. Saturable binding occurred only in the presence of 100nm NaCl. Equimolar concentrations of other chloride salts, eg choline chloride, potassium chloride, rubidium chloride, tris hydrochloride and cesium chloride, failed to support mazindol-sensitive [H]GBR-12935 binding. Furthermore, these cations inhibited [H]GBR-12935 binding demonstrating the high specificity of binding for Na+. In contrast, [H]GBR-12935 binding had no apparent anion requirement.

The effect of NaCl on mazindol-sensitive [H]GBR-12935 binding to canine striatal membranes was concentration dependent. Figure 1 illustrates the sigmoidal behaviour of [H]GBR-12935 binding as a function of Na+ concentration. Analysis of the data using the Hill equation revealed a Hill coefficient of 1.9 ± 0.6 consistent with the hypothesis that two sodium ions are required for the binding of each molecule of [H]GBR-12935. Half maximal stimulation of specific [H]GBR-12935 binding by Na+ was observed at 13.9 ± 4.8 mM (n=4). Further experiments showed that Na+ increased the affinity of mazindol-sensitive [H]GBR-12935 binding with no effect on the maximal number of binding sites.

Fig. 1 Dependence of mazindol-sensitive [H]GBR-12935 binding on Na+ concentration. Canine striatal membranes were incubated in 5nm bicarbonate/phosphate buffer pH 7.3 for 1 hour at 22°C, with [H]GBR-12935 (final concentration 1.0mM) in the presence of increasing sodium. Non specific binding was measured in the presence of 3mM mazindol and subtracted from the total [H]GBR-12935 binding. Data are mean ± SD values from a single experiment that was repeated with similar results.

In conclusion, these results suggest that the binding of [H]GBR-12935 to the dopamine transporter in canine striatum is Na+-dependent but Cl-independent. Furthermore, the requirement of two Na+ ions for ligand binding and the finding that Na+ effects the Kd for [H]GBR-12935 binding but not the Bmax is consistent with the binding of other inhibitors to amine transporter carriers, such as the 5-hydroxytryptamine transporter [8].

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