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# Murine lymphocytes lack clearly defined receptors for muscarinic and dopaminergic ligands

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[$^3$H]Quinuclidinyl benzilate and [$^3$H]spiperone binding to murine lymphocytes is displaceable but differs from binding to brain receptor sites for these ligands: (1) binding to intact lymphocyte preparations was not saturable; (2) disruption of intact lymphocytes was associated with a marked loss of displaceable ligand binding; (3) drugs differentially displace these ligands in lymphocytes compared to brain. Displaceable binding was increased following incubation of lymphocytes under phospholipid methylating conditions; however, marked effects on cell viability and cell recovery make it difficult to interpret these binding changes. If dopaminergic and cholinergic receptors do exist on lymphocytes, their binding characteristics are profoundly different from comparable cns receptors.

We have noted papers in recent years describing dopaminergic and muscarinic binding sites on mammalian lymphocytes. Uzan et al (1981) characterized [$^3$H]spiperone binding in murine lymphocytes with apparent B-cell specificity. Binding was stereospecific, saturable (Kd ~ 5 nM) and showed time course dissociation characteristics consistent with a 'classic' pharmacological receptor (Uzan et al 1981). Further, those authors reported data (Le Fur et al 1981) suggesting a coupling between the 'dopaminergic' receptors and phospholipid methylation.

Several authors (Zalcman et al 1981; Strom et al 1981; Gordon et al 1978) describe muscarinic cholinergic binding sites on human and murine lymphocytes. These sites are T-cell specific and saturable (Kd 10-100 nM) and are retained in homogenized tissue preparations (Bidart et al 1983).

Maloteaux et al (1982) question the existence of both dopamine and muscarinic receptors on human lymphocytes. Those authors report that the potencies of various drugs in displacing labelled ligands differ markedly between human lymphocyte and rat striatal preparations. They hypothesize that labelled ligand is entrapped within cellular compartments of lymphocytes and may be mistakenly interpreted as 'specific binding'.

Because of our interest in both of these receptors and the interactive role of phospholipids on receptor function, we have studied [$^3$H]spiperone ([$^3$H]SPD) and [$^3$H]quinuclidinyl benzilate ([$^3$H]QNB) binding in murine lymphocytes. Based on our data to date, we share some of the reservations of Maloteaux et al and similarly question whether the binding data clearly support the existence of receptors. We wish to briefly report our observations.

*Methods and results*

Spleens were collected from male Swiss-albino mice (20–30 g). All dilutions were performed with Hank's balanced salt solution and lymphocytes isolated by means of a Ficoll gradient procedure (cf. Uzan et al 1981). Cells were washed twice with Hank's solution and counted. Viability of lymphocytes (erythrocin B exclusion) exceeded 97%.

In-vitro 'binding' assays were performed using 1–3 × $10^6$ cells/tube and either [$^3$H]SPD (specific activity—21·0 Ci mmol$^{-1}$) or [$^3$H]QNB (specific activity—30·2 Ci mmol$^{-1}$) in a total volume of 1·0 ml. Incubations were for 1 h at 37 °C for [$^3$H]SPD binding and at 25 °C for [$^3$H]QNB binding. 'Specific binding' was defined in the presence or absence of $10^{-5}$ M haloperidol or $10^{-4}$ M atropine in the SPD and QNB binding respectively.

In order to address the 'binding'/'entrapment' issue we examined both intact and particulate tissue preparations. Particulate tissue preparations were prepared by Polytron action (setting 5; 2 × 30 s bursts).

67% of total SPD binding and 33% of total QNB binding was found to be displaceable. We were unable to achieve saturation in intact lymphocytes for either [$^3$H]SPD (up to 5000 nM) or [$^3$H]QNB (up to 250 nM). In

* Correspondence.

a particulate lymphocyte preparation, [$^3$H]SPD binding was non-saturable; [$^3$H]QNB binding saturated with a Kd of 25 nM; Bmax of 2·8 pmol $mg^{-1}$ protein (these data are similar to those published by Bidart et al 1983). To determine if differences in mouse strain or sex could account for differences seen by us and others, lymphocytes from female Balb-c and CD-1 mice (20–24 g) were also examined. Again, using intact lymphocyte preparations, we were unable to achieve saturation for either ligand up to a concentration of 250 nM. Parallel assays of rat cortical and striatal synaptosomes for both ligands using identical conditions as for lymphocytes, yielded binding constants consistent with previously published results.

Specific binding of both ligands was reduced by 80% in lymphocytes that had been osmotically lysed (30 min in Hank's solution diluted 1 : 10), and by 50–80% in polytron disrupted tissue. Freezing abolished all specific binding.

No such changes were seen with synaptosomes.

To determine if these binding sites behaved comparably to receptors in other tissue preparations, we investigated the possible interaction of phospholipid methylation with receptor regulation. Intact lymphocytes were incubated at 4 °C for 24 h with $5 \times 10^{-2}$ to $5 \times 10^{-4}$ M L-methionine as described by Le Fur et al (1981) and then assayed for both [$^3$H]SPD and [$^3$H]QNB binding. In lymphocytes pre-incubated with methionine, specific binding was generally increased by 40 to 500% for both ligands. Our most consistent findings, however, were marked reductions in cell viability (80–100% loss of viability) and cell recovery (haemocytometric cell counts reduced by 50%) after the 24 h incubation with L-methionine (control conditions without methionine showed no decrease in viability or number).

We attempted to reduce cell damage and loss by reducing pre-incubation time to 1 h at 37 °C. Cell viability and total number remained constant but the effects of L-methionine on specific binding for both ligands were slight and variable (0–30% above controls). Preincubation of particulate lymphocyte preparations with *S*-adenosyl-L-methionine (200 μM) for 1 h at 37 °C did not affect specific binding of either ligand.

*Discussion*

These data raise a number of questions concerning the binding of QNB and SPD in lymphocytes. First, is this binding indeed 'specific' with regard to a true receptor or merely reflective of another process? Our inability to achieve saturation with either ligand in intact lymphocytes caused us to consider other possibilities for concentration dependent (but non-saturable) increases of displaceable ligand binding. Cellular uptake which might account for this is usually envisioned as passive (i.e. diffusion) or active (energy dependent). Passive uptake was considered less likely in view of the displaceability of binding by unlabelled ligand. We attempted to assess uptake via disruption of lymphocyte membranes and found a marked reduction in 'specific' binding. In light of these data we speculate that perhaps an element of the 'specific' binding observed in intact lymphocytes is in fact ligand entrapped by an uptake process. Difficulties with this explanation arise when we consider why lymphocytes might have an uptake site for these ligands and why, after an apparent reduction in both cell number and viability in methionine pre-incubated lymphocytes, 'specific' binding increases. We can offer no ready explanation. Alternatively, the loss of binding seen in disrupted, lysed or frozen tissue might result from a highly labile receptor protein which is easily dissociated from the membrane.

The increases in SPD binding in lymphocytes pre-incubated for 24 h with methionine must be cautiously interpreted in light of the marked reductions in cell number and viability at the time of assay. Is increased binding indeed a true effect of phospholipid methylation or merely a reflection of morphologic and biochemical changes associated with cell death?

It should be pointed out that the mononuclear cells collected by the Ficoll gradient technique represent a diverse cell population and that different cell types have been reported to show different binding characteristics. Thus, our data from a mixed lymphocyte population may differ from those of others using more homogeneous cell lines. (N.B. – Initial reports of QNB and SPD binding in lymphocytes were based on studies with mixed cell populations.)

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