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# COMMUNICATIONS

## Selective labelling of murine B lymphocytes by [$^3$H]spiroperidol

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Recently we have located [$^3$H]spiroperidol binding sites in a mixture of blood mononuclear leucocytes separated by Ficoll-Paque gradient centrifugation (Le Fur et al 1980) but the presence of T and B lymphocytes as well as monocytes (Zucker Franklin 1974) in this preparation raises the question of what kind of mononuclear leucocytes are really concerned with these binding sites. Such a study might be a valid preliminary approach to the understanding of the possible role of dopaminergic receptors in the cellular-mediated immune response. We now report on more purified cell preparations isolated from blood, spleen, thymus or peritoneal exudates.

Female CD1 (from Charles River) Balb/C and Nu/Nu mice (20 ± 2 g) (from Ifa-Credo) were used. Lymphocytes were isolated from fresh heparinized blood of spleen using a Ficoll-Paque gradient and hypotonic lysis of erythrocytes (Boyum 1967; Fotino et al 1971). T lymphocytes were obtained after a passage of the cells over columns of glass wool (Pattengale et al 1974). A monocyte-enriched population was obtained by passage over Sephadex G-10 columns then mechanical agitation (Ly & Mishell 1974). Polymorphonuclear leucocytes were prepared by using a sedimentation in a high molecular weight dextran (Galant et al 1978). Macrophages were obtained from peritoneal exudates (Fray et al 1979), red blood cells after a passage over a cellulose column (Beutler et al 1976), platelet suspension according to the method of Vargaftig et al (1976) and thymocytes according to Ford & Hunt (1973).

Viability of the cells was determined by exclusion of trypan blue dye and was always greater than 95%. The cells were resuspended in Hank's balanced salt solution. Specific binding was determined as previously described (Le Fur et al 1980) by incubating in triplicate $10^6$ cells at 37 C in 1 ml of Hank's salt solution with 5 nM of [$^3$H]spiroperidol (25·1 Ci mmol$^{-1}$ NEN) in the presence (non-specific binding) or absence (total binding) of 10 $\mu$M haloperidol. Cells were filtered after 60 min using Whatman GF/B filters which were then rinsed 3 times with 4 ml of Hank's solution, dried and the radioactivity measured by liquid scintillation spectrometry. Specific binding was 65 to 70% of the total binding.

The B and T mixture of intact lymphocytes isolated from CD1 mouse spleen have a specific [$^3$H]spiroperidol binding that was linear from $10^4$ to $10^7$ cells. Therefore all assays were routinely made with $10^6$ cells.

[$^3$H]Spiroperidol binding was temperature-dependent with a maximum binding at 37 °C. The [$^3$H]spiroperidol binding was saturable, whereas the non-specific binding increased linearly with [$^3$H]spiroperidol concentration. A Scatchard analysis of the [$^3$H]spiroperidol specific binding indicated a single binding component within the concentration range used. The apparent dissociation constant ($K_D$) was 4·8 ± 0·2 nM. The total number of binding sites ($B_{max}$) was 115 ± 5 f mol/$10^6$ cells, which corresponds to 60 000 [$^3$H]spiroperidol binding sites per cell (Table 1). Hill analysis gave a Hill coefficient ($n_H$) of 0·65 ± 0·05 suggesting the presence of negative cooperative effects or heterogenous binding sites within the concentration range studied. [$^3$H]Spiroperidol binding reached equilibrium relatively rapidly in this cell preparation (Fig. 1) giving a rate constant for association of $K_1$ = 0·0024 nM min$^{-1}$ at 37 °C. Moreover [$^3$H]spiroperidol dissociation followed first order kinetics, giving a rate constant for dissociation of $K_2$ = 0·012 min$^{-1}$ at 37 °C.

Finally the dissociation constant ($K_D = K_2/K_1$) based on these direct kinetic data was 5 nM which corresponds closely to the $K_D$ estimated from equilibrium studies (4·8 nM).

All the antidopaminergic drugs tested were effective inhibitors of [$^3$H]spiroperidol binding. Their $K_i$ values correlated linearly and significantly ($r = 0·968$, $P < 0·001$) with those found in rat brain striatum. [$^3$H]Spiroperidol binding was stereospecific since (+)-butaclamol was 400 times more potent than its stereoisomer (—)-butaclamol. Moreover apomorphine and dopamine were effective inhibitors at micromolar concentrations whereas noradrenaline, 5-HT or the anti-5-HT drug methysergide were inactive. The Hill coefficient was for all tested drugs lower than 1.

All those data concerned the mixture of T and B lymphocytes. However in other experiments, lymphocytes were separated either by passage of the Ficoll-Paque gradient preparation over column of glass

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Table 1. Kinetic constants of high affinity [$^3$H]spiroperidol binding to lymphocytes from spleen of different female mouse strains. Each value was the mean of 2 to 4 determinations by using Scatchard ($K_D$ and $B_{max}$) and Hill ($n_H$) plots. The concentrations of [$^3$H]spiroperidol used were 0·1; 0·5; 1; 2; 3; 4; 5; 6; 7 nM.

| Mouse strains | $K_D$ (in nM) | $B_{max}$ (in fmol/$10^6$ cells) | $n_H$ (Hill coeff.) |
|---|---|---|---|
| CD1 | 4·8 ± 0·2 | 115 ± 5 | 0·65 ± 0·05 |
| Balb/C | 4·9 ± 0·5 | 88 ± 7 | 0·74 ± 0·04 |
| Nu/Nu | 5·7 ± 0·4 | 108 ± 4 | 0·75 ± 0·04 |

wool, or by isolation from mouse thymus (CD1 or Balb/C). In such T purified preparations no [$^3$H]-spiroperidol binding was detectable. Elsewhere no binding was detectable in blood platelets, red blood cells, macrophages, polynuclear leucocytes or monocytes (data not shown),

Therefore [$^3$H]spiroperidol binding appeared to be selective for B lymphocytes. Moreover good binding occurred with Nu/Nu mice spleen lymphocytes which contain only B lymphocytes (Table 1). This specific binding fulfils the criteria expected for a ligand interaction with a dopaminergic receptor, particularly with good stereospecificity (Table 2).

Finally our data suggest that [$^3$H]spiroperidol binding could be a selective marker for B lymphocytes, so lymphocytes might be a useful clinical readily accessible model for detecting human pathological changes in dopaminergic receptors. Dopaminergic binding sites on B lymphocytes could also initiate further studies concerning their role in the lymphocyte-mediated immune response.

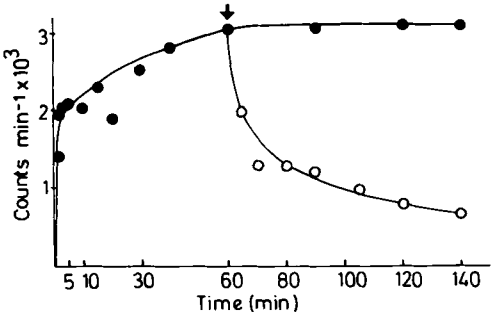


FIG. 1. Time course of [$^3$H]spiroperidol binding to lymphocytes from CD1 mouse spleen. Association of [$^3$H]spiroperidol to mouse lymphocytes was measured under the standard assay conditions as a function of time from the addition of [$^3$H]spiroperidol (5 nM) (●). The non-specific binding (with 10 μM haloperidol added) was subtracted from the total binding (without haloperidol) to give specific binding. At the time indicated by the arrow (60 min) 10 μM of haloperidol was added to prevent the rebinding of dissociated [$^3$H]spiroperidol (○). The samples were incubated for varying amounts of time and specific [$^3$H]spiroperidol binding assayed as described in methods. Each point is the mean of 4 to 6 determinations; standard errors were less than 5%.

Table 2. Inhibition of [$^3$H]spiroperidol binding to mouse lymphocytes. Displacement of [$^3$H]spiroperidol by various agents was effected under standard conditions of binding assay as described in the methods except that NA, DA, 5-HT were tested in the presence of 10 μM pargyline. Serial dilutions (4 to 6) in triplicate were added to the binding assay to estimate IC50 values (concentration causing 50% inhibition of [$^3$H]spiroperidol binding. $K_I = IC50/1 + S/K_D$; C = [$^3$H]spiroperidol concentration = 5 nM; $K_D$ = affinity constant = 4·8 nM and 5·7 in the CD1 and Nu/Nu mice respectively.

| | CD1 Mice | | Nu/Nu Mice | |
|---|---|---|---|---|
| | $K_I$ (in nM) | $n_H$ (Hill coeff.) | $K_I$ (in nM) | $n_H$ (Hill coeff.) |
| Pimozide | 2 | 0·3 | 3 | 0·3 |
| Haloperidol | 3 | 0·2 | 6 | 0·2 |
| UK 177 | 4 | 0·7 | 11 | 0·3 |
| (+)-Butaclamol | 6 | 0·4 | 16 | 0·3 |
| Chlorpromazine | 10 | 0·4 | 21 | 0·4 |
| Mezilamine | 98 | 0·2 | 86 | 0·2 |
| Clozapine | 221 | 0·4 | 160 | 0·3 |
| (−)-Butaclamol | 2460 | 0·3 | 2140 | 0·2 |
| Sulpiride | 3440 | 0·3 | 3210 | 0·4 |
| Apomorphine | 885 | 0·4 | 2940 | 0·4 |
| Dopamine | 690 | 0·5 | 1340 | 0·3 |
| Noradrenaline | 39 500 | 0·2 | 29400 | 0·4 |
| 5-HT | 30 000 | 0·2 | 37500 | 0·5 |
| Methysergide | 9830 | 0·6 | 8020 | 0·6 |

## REFERENCES

Beutler, F., West, C., Blum, R. G. (1976) J. Lab. Clin. Med. 88: 328–333

Boyum, A. (1967) Scand. J. Clin. Lab. Invest. 97 (Suppl): 77–89

Ford, W. L., Hunt, S. V. (1973) in: Wier, D. (ed.) Handbook of Experimental Immunology, Cellular Immunology. 1–27, Blackwell Scientific Publications, Oxford

Fotino, M., Merson, E. J., Allen, F. H. (1971) Ann. Clin. Lab. Sci. 1: 131–133

Fray, A., Lorinet, A. M., Halpern, B. (1979) Ann. Immunol. (Institut Pasteur) 130C: 367–372

Galant, S. P., Underwood, S., Duriseti, L., Insel, P. A. (1978) J. Lab. Clin. Med. 92: 613–618

Le Fur, G., Phan, T., Uzan, A. (1980) Life Sci. 26: 1139–1148

Ly, I., Mishell, R. I. (1974) J. Immunol. Meth. 5: 239–248

Pattengale, P. K., Smith, R. W., Gerber, P. (1974) J. Natl. Cancer. Inst. 52: 1081–1086

Vargaftig, B. B., Tramier, Y., Chignard, M. (1976) Prostaglandins 8: 328–333

Zucker Franklin, D. (1974) J. Immunol. 112: 234–240