ELSEVIER

European Journal of Pharmacology 301 (1996) 189–194

ejp

Pharmacological characterisation of Ca^{2+} channels of the L-type in human peripheral blood lymphocytes

Alberto Ricci [a], Alberto Bisetti [a], Elena Bronzetti [b], Laura Felici [b], Fabio Ferrante [a], Franco Veglio [c], Francesco Amenta [d,*]

[a] *Dipartimento di Scienze Cardiovascolari e Respiratorie, Università 'La Sapienza', Sezione Malattie Apparato Respiratorio, Rome, Italy*
[b] *Sezione di Morfologia Sperimentale, Rome, Italy*
[c] *Cattedra di Medicina Interna, Ospedale San Vito, Università di Torino, Turin, Italy*
[d] *Sezione di Anatomia Umana, Istituto di Farmacologia, Università di Camerino, Camerino, Italy*

Received 21 December 1995; accepted 3 January 1996

Abstract

Ca^{2+} channels of the L-type were characterised in intact human peripheral blood lymphocytes using a radioligand binding technique and the dihydropyridine-type Ca^{2+} channel antagonist [^{3}H](+)-PN 200-110 (isopropyl-4-(2,1,3-benzoxadiazol-4-yl)1,4-dihydro-5-methoxycarbonyl-2,6-dimethyl-3-pyridine carboxylate) as a ligand. [^{3}H](+)-PN 200-110 binding to human peripheral blood lymphocytes was time-, temperature-, concentration-dependent and of high affinity. The dissociation constant (K_d) value was 0.4 ± 0.02 nM and the maximum binding capacity (B_{max}) was 33.5 ± 1.6 fmol/10^6 cells. Pharmacological analysis of [^{3}H](+)-PN 200-110 binding to human peripheral blood lymphocytes was consistent with the labelling of a Ca^{2+} channel of the L-type. In fact, dihydropyridine derivatives were the most potent competitors of [^{3}H](+)-PN 200-110 binding, whereas phenylalkylamine and benzothiazepine compounds or non-selective Ca^{2+} channel modulators were weak or ineffective displacers. These findings are the first observation that human peripheral blood lymphocytes express Ca^{2+} channels of the L-type. The possibility that Ca^{2+} channel antagonists may interfere with immune system function is discussed.

Keywords: Lymphocyte; Ca^{2+} channel; PN 200-110; Radioligand binding; Immune function; (Human)

1. Introduction

Ca^{2+} channels represent the gate for regulating Ca^{2+} entry through the external cellular membrane (Fleckenstein, 1983; Schwartz and Triggle, 1984; Kokobun et al., 1986). Functional, pharmacological and clinical evidence suggests an important role of compounds active on Ca^{2+} channels in the control of excitation, contraction, impulse propagation and metabolism of different tissues. Ca^{2+} channel blockers (commonly defined as Ca^{2+} channel antagonists) are compounds able to block with high affinity Ca^{2+} channel binding sites and represent an interesting class of therapeutic agents used primarily in the treatment of cardiovascular and neurological disorders (Nayler, 1988; Scriabine et al., 1989; Spedding and Paoletti, 1992).

Voltage-dependent Ca^{2+} channels were classified into various types on the basis of functional, radioligand binding, autoradiographic and molecular biology evidence. They include L-type (long lasting) channels, T-type (transient) channels, N-type (neuronal) channels, P-type (Purkinje), R-type and Q-type channels (Schwartz and Triggle, 1984; Triggle, 1991; Spedding and Paoletti, 1992). Blockers of Ca^{2+} channels of the L-type have assumed therapeutic significance, as compounds active in the treatment of hypertension, angina pectoris and more recently of cerebrovascular disorders (Nayler, 1988; Scriabine et al., 1989; Triggle, 1991). Dihydropyridine derivatives which bind the α_1-subunit of Ca^{2+} channel of the L-type display a high affinity for vascular smooth muscle Ca^{2+} channels and represent a family of powerful anti-vasoconstrictors (Triggle, 1991; Spedding and Paoletti, 1992).

The application of radioligand binding techniques to the analysis of dihydropyridine-sensitive Ca^{2+} channels has allowed the characterisation of several biochemical, pharmacological and molecular properties of Ca^{2+} channel of

* Corresponding author. Istituto di Farmacologia, Via Scalzino 5, 62032 Camerino, Italy. Fax: +39-737-630219.

PII S0014-2999(96)00016-7

the L-type (Glossmann and Ferry, 1985; Spedding and Paoletti, 1992). The majority of these studies was performed on brain, vascular and non vascular smooth muscle and cardiac tissue (Glossmann et al., 1982; Glossmann and Ferry, 1985; Spedding and Paoletti, 1992; Ferrante and Amenta, 1993). In contrast, as far as we know, no studies were made on the expression of Ca^{2+} channels by the immune system. Human peripheral blood lymphocytes could represent an easily obtainable human cell population for analysing interactions between Ca^{2+} channels and immune system. It is known that lymphocytes express receptors for neurotransmitters, neurohormones and immunotransmitters (Bishopric et al., 1980; Wybran, 1986; Maslinski, 1989; Nagai et al., 1993; Takahashi et al., 1992; Ricci and Amenta, 1994). The present study was designed to investigate by the use of radioligand binding techniques if human peripheral blood lymphocytes express Ca^{2+} channels of the L-type.

2. Materials and methods

2.1. Peripheral blood lymphocytes preparation

Thirty milliliters of venous blood were sampled from 10 healthy human adult volunteers (age range 25–35 years). Blood was collected in plastic tubes containing heparin as anticoagulant and diluted with an equal volume of 0.9% NaCl saline solution. Six milliliters of diluted blood were layered upon 3 ml of Lymphoprep (Nycomed Pharma, Oslo, Norway), being careful to avoid mixture between the two fluids.

The sample was centrifuged at $800 \times g$ for 20 min at room temperature using a swing-out rotor. At the end of centrifugation, mononuclear cells were layered in a distinct band at the sample/medium interface. Cells were then collected using a Pasteur pipette without removing the upper layer. Harvest cells were re-suspended in a saline solution and re-centrifuged at $250 \times g$ for 10 min at room temperature. With this procedure erythrocyte contamination was less than 10%, whereas washing procedures removed most of the platelets. Monocytes were eliminated by incubating cells in plastic tissue culture flasks for 60 min at 37°C under 5% CO_2 and 95% air. The non-adherent cell population was re-suspended in saline solution at a final concentration of 10^6 cells/ml. Further details are reported in a recent paper of our group (Ricci and Amenta, 1994).

2.2. Radioligand binding experiments

Three hundred microliters of the 10^6 cell preparation were incubated for different times (5, 30, 60, 90 and 120 min) and temperatures (4°C, 25°C and 37°C) in a 170 mM Tris HCl buffer (pH 7.4) in the presence of increasing concentrations (0.125–2 nM) of [^{3}H](+)-PN 200-110 (isopropyl-4-(2,1,3-benzoxadiazol-4-yl)-1,4-dihydro-5-methoxycarbonyl-2,6-dimethyl-3-pyridine carboxylate). Non-specific binding was defined by incubating sections with [^{3}H](+)-PN 200-110 plus 1 μM nifedipine. At the end of the incubation, cells were washed with the incubation medium after isolation onto Whatman GF-B glass fibre filters, by a marifold filtration apparatus. The filters were then transferred into scintillation vials containing 4 ml of scintillation solution and counted in a Packard liquid scintillation spectrometer at an efficiency of 40%.

The pharmacological specificity of [^{3}H](+)-PN 200-110 binding to human peripheral blood lymphocytes was assessed by incubating cells at 25°C for 60 min with 0.5 nM [^{3}H](+)-PN 200-110 in the presence of increasing concentrations of compounds active on Ca^{2+} channels such as dihydropyridines, phenylalkylamine and benzothiazepine derivatives and non-selective Ca^{2+} channel antagonists. Incubation conditions of competition experiments were chosen so as to allow the development of the highest specific/non-specific binding ratio. After incubation cells were processed as described above.

2.3. Data analysis

Data from binding experiments were calculated by linear regression analysis of Scatchard plot saturation isotherms. Inhibition constant (K_i) values were calculated with the method of Cheng and Prusoff (1973), considering as IC_{50} the displacer concentration inhibiting 50% of specific binding. Hill numbers were calculated with the formula $B_0 - B$/vs. log concentration, where B_0 and B were specific binding in the absence and presence of competitor, respectively.

2.4. Chemicals

[^{3}H](+)-PN 200-110 (specific activity 80 Ci/mmol) was obtained from Amersham Radiochemical Centre (Buckinghamshire, UK). Nicardipine hydrochloride and isomers of PN 200-110 were obtained by Sandoz Prodotti Farmaceutici (Milan, Italy). Other compounds tested were purchased from Research Biochemicals (Natick, MA, USA) and from Merck (Darmstadt, Germany).

3. Results

[^{3}H](+)-PN 200-110 was bound specifically to human peripheral blood lymphocytes. The binding was time- (Fig. 1) and temperature-dependent (Fig. 1). The plateau of specific binding was reached at 60 min of incubation and at the temperature of 25°C (Fig. 1). A standard time of 60 min and a temperature of 25°C were used in subsequent binding experiments. The binding of [^{3}H](+)-PN 200-110 to human peripheral blood lymphocytes was concentration-dependent (Fig. 2A), belonging to a single class of high affinity sites (Fig. 2B). The dissociation constant

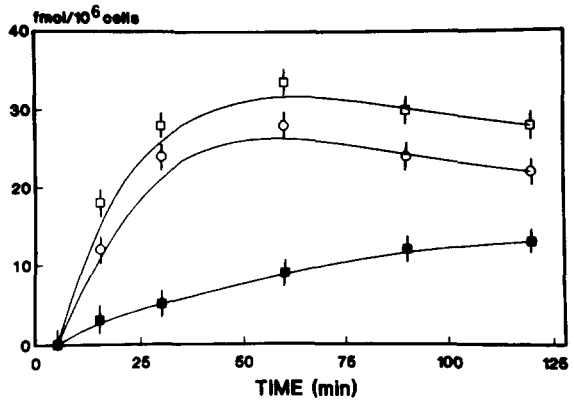

Fig. 1. Influence of different incubation times and temperatures (4°C, ■; 25°C, □; and 37°C, ○) on specific [^{3}H](+)-PN 200-110 binding (abscissa) to human peripheral blood lymphocytes. The points are the means ± S.E.M. of triplicate determinations.

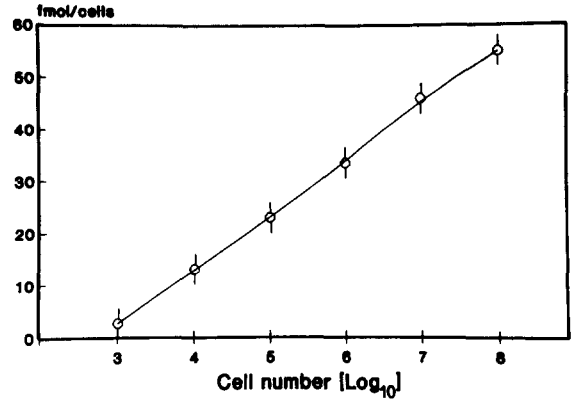

Fig. 3. Specific [^{3}H](+)-PN 200-110 binding as a function of human peripheral blood lymphocyte number. Cells were incubated with a 0.5 nM radioligand concentration. Binding experiments were performed as described in the materials and methods section. The points are the means ± S.E.M. of triplicate determinations.

value (K_d) value was 0.4 ± 0.02 nM and the maximum binding density of binding sites (B_{max}) was 33.5 ± 1.6 fmol/10^6 cells (Fig. 2A,B). Using a radioligand concentration of 0.5 nM, a value close to the K_d (Fig. 2A), approximately 65% of [^{3}H](+)-PN 200-110 was bound specifically (Fig. 2A). A concentration of 0.5 nM was therefore used in standard binding experiments. The number of [^{3}H](+)-PN 200-110 binding sites augmented as a function of cell number increase (Fig. 3).

Data of pharmacological specificity of [^{3}H](+)-PN 200-110 binding to human peripheral blood lymphocytes are shown in Fig. 4 and summarized in Table 1. As can be seen, dihydropyridine compounds tested were the most powerful competitors of [^{3}H](+)-PN 200-110 binding to human peripheral blood lymphocytes. The binding was also stereo specific since the dextro-rotatory form of unlabelled PN 200-110 was approximately 15 times more potent than the levo-rotatory form (Table 1). The phenylalkylamine (verapamil) and benzothiazepine (diltiazem) derivatives and the non-selective Ca^{2+} channel antagonists tested (cinnarizine and flunarizine) were less powerful or

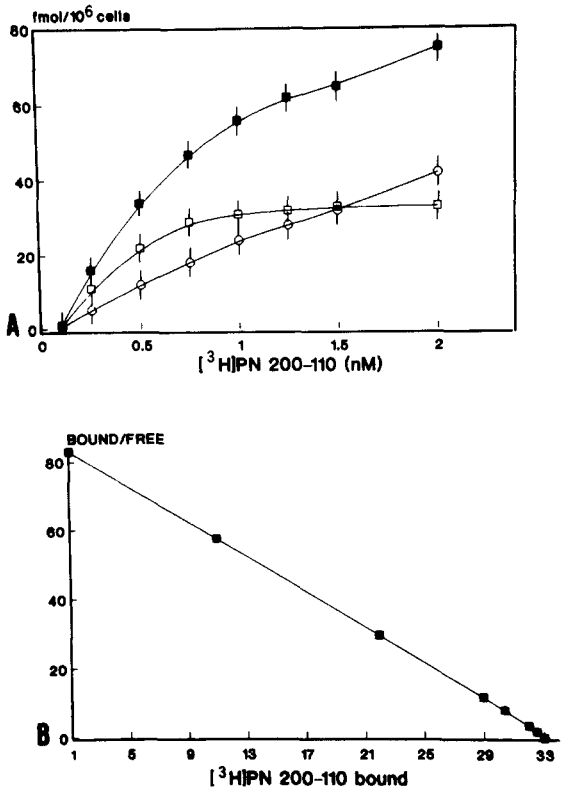

Fig. 2. A: Saturation curve of [^{3}H](+)-PN 200-110 binding to human peripheral blood lymphocytes. Cells were incubated with increasing concentrations of the radioligand alone (total binding, ■) or plus 1 μM nifedipine to define non-specific binding (○). Specific binding (□) was obtained by subtracting non-specific from total binding. The points are the means ± S.E.M. of triplicate determinations. B: Scatchard analysis of specific [^{3}H](+)-PN 200-110 binding to human peripheral blood lymphocytes. The B_{max} value was 33.5 ± 1.6 fmol/10^6 cells. Points are means of triplicate determinations. The standard error was less than 10%.

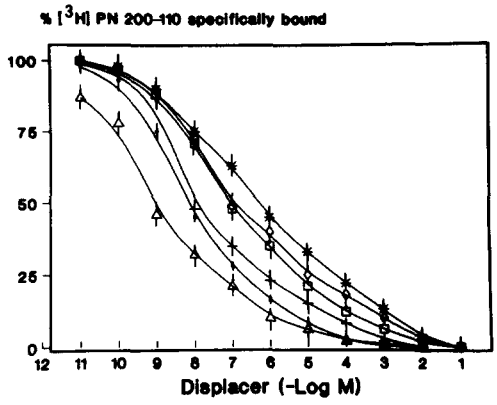

Fig. 4. Displacement of specific [^{3}H](+)-PN 200-110 binding to human peripheral blood lymphocytes by increasing concentrations of compounds active on Ca^{2+} channels. Cells were incubated with a 0.5 nM radioligand concentration in the presence of increasing concentrations of (+)-PN 200-110 (△), nicardipine (●), nifedipine (†), verapamil (□), BAY&K 8644 (◇) and diltiazem (*). The points are the means ± S.E.M. of triplicate determinations.

Table 1
Pharmacological specificity of [^{3}H](+)-PN 200-110 to human peripheral blood lymphocytes

Compound	K_i (nM)	nH
BAY&K 8644	40.3 ± 2.5	1.2 ± 0.04
Cinnarizine	> 5000	n.d.
Diltiazem	280 ± 18	2.1 ± 0.07
Flunarizine	> 5000	n.d.
Nicardipine	2.85 ± 0.1	1.0 ± 0.05
Nifedipine	4.21 ± 0.2	1.0 ± 0.03
Nimodipine	6.19 ± 0.3	1.0 ± 0.05
(+)-PN 200-110	0.53 ± 0.01	1.0 ± 0.02
(−)-PN 200-110	7.95 ± 0.3	1.1 ± 0.03
Verapamil	35 ± 1.6	1.5 ± 0.08

Values represent the competitor dissociation constant (K_i) determined according to the method of Cheng and Prusoff (1973). The data are the means ± S.E. of three to five experiments performed in triplicate. Hill numbers (nH) were calculated as described in section 2.3. n.d. = not determinable.

ineffective competitors of [^{3}H](+)-PN 200-110 binding (Table 1). Both verapamil and diltiazem displaced [^{3}H](+)-PN 200-110 binding at concentrations significantly higher than dihydropyridine-type Ca^{2+} channel antagonists (Fig. 4). Verapamil and diltiazem inhibited completely radioligand binding at a concentration of 0.1 and 1 M, respectively (Fig. 4).

4. Discussion

Functional studies have demonstrated a primary role of Ca^{2+} in lymphocyte activation and transformation and binding of Ca^{2+} to lymphocyte membrane is the first early transmembrane process involved in cell activation (Kay, 1971; Whitney and Sutherland, 1972; Resch et al., 1978; Freedman et al., 1981). Compounds able to suppress lymphocyte proliferation interfere with Ca^{2+} influx. This suggests that Ca^{2+} entry into lymphocytes represents a mechanism leading to cellular activation (Lewis and Cahalan, 1990). The maintenance of a constant intracellular and extracellular Ca^{2+} concentration seems to be a fundamental factor in regulating cell function which is in part controlled by Ca^{2+} channels embedded in plasma membrane (Lewis and Cahalan, 1990).

As mentioned in the Introduction, cardiac and smooth muscle cell relaxing effects of Ca^{2+} channel blockers are documented as well as the use of these compounds in the treatment of hypertension, angina and central nervous system diseases including cerebral ischemia, vascular dementia and epilepsy (Nayler, 1988; Scriabine et al., 1989; Triggle, 1991). In contrast, the effects of these compounds on immune functions are controversial.

It has been reported that Ca^{2+} channel blockers may modulate lymphocyte activity in two ways. Low concentrations of these compounds generate uniform modulation of transmembrane electrical potential (depolarisation) of lymphocytes demonstrated by both in vitro and in vivo studies. Depolarisation is the first step in cell adhesion and promotes responses to mitogens. High concentrations of Ca^{2+} channel antagonists inhibit responses to mitogens (Dugas et al., 1986; Kunert-Radek et al., 1990; Lewis and Cahalan, 1990; Morgano et al., 1990; Weir, 1991; Chitwood and Heim-Duthoy, 1993).

Clinical pharmacology studies have suggested that Ca^{2+} channel blockers may inhibit transiently lymphocyte functions in vivo (Morgano et al., 1990). However, the effects of inactive and active forms of Ca^{2+} channel blockers on peripheral blood mononuclear cells were similar, suggesting that immune effects of these compounds may be unrelated to the inhibition of transmembrane Ca^{2+} influx (Weir, 1991; Weir et al., 1992). In view of this it seemed to us of some interest to identify if human peripheral blood lymphocytes express Ca^{2+} channels of the L-type which are rather sensitive to pharmacological manipulation (Nayler, 1988; Spedding and Paoletti, 1992).

The development in the last 10–15 years of selective ligands for Ca^{2+} channels allowed the pharmacological analysis and more recently the molecular characterization of Ca^{2+} channels in several tissues such as brain, heart, skeletal muscle, vascular, intestinal and urinary smooth muscle (Glossmann et al., 1982; Murphy and Snyder, 1982; Bolger et al., 1983; Cortes et al., 1983; Cortes et al., 1984; Glossmann and Ferry, 1985; Godfraind et al., 1988; Morel and Godfraind, 1988; Wibo et al., 1988; Yamada et al., 1988; Slish et al., 1989; Latifpour et al., 1992; Ferrante and Amenta, 1993). Radiolabelled dihydropyridines are the Ca^{2+} channel blockers more used in binding experiments and [^{3}H](+)-PN 200-110 is probably the radioligand most utilised. It labels brain and skeletal and smooth muscle Ca^{2+} channels of the L-type with affinity constant between 0.1 and 12 nM (Ferry et al., 1983; Glossmann and Ferry, 1985; Wibo et al., 1988). The K_d value of [^{3}H](+)-PN 200-110 in our binding experiments with human peripheral blood lymphocytes is in the range of affinity values found by other investigators for Ca^{2+} channels of the L-type (see Glossmann et al., 1982; Spedding and Paoletti, 1992). This suggests that [^{3}H](+)-PN 200-110 binding sites expressed by peripheral blood lymphocytes are similar to Ca^{2+} channels of the L-type present in other tissues. Further support to the hypothesis of labelling Ca^{2+} channel of the L-type in peripheral blood lymphocytes comes from competition binding experiments in which dihydropyridine-type compounds were the most powerful competitors of [^{3}H](+)-PN 200-110 binding, with Hill numbers near unit. Phenylakylamine (verapamil) and benzothiazepine (diltiazem) Ca^{2+} channel blockers were less powerful competitors of [^{3}H](+)-PN 200-110 binding, whereas non-selective Ca^{2+} channel antagonists (cinnarizine and flunarizine) were without effect on [^{3}H](+)-PN 200-110 binding. This pharmacological profile strongly suggests that in our radioligand binding experiments we have labelled a Ca^{2+} channel of the L-type

(dihydropyridine-sensitive) (Glossmann and Ferry, 1985; Spedding and Paoletti, 1992). The observation that verapamil and diltiazem competed to some extent [^{3}H](+)-PN 200-110 binding suggests the occurrence of an allosteric interaction between dihydropyridine-sensitive and phenylalkylamine- or benzothiazepine-sensitive sites in human peripheral blood lymphocytes similarly as found in vascular smooth muscle (Ferrante and Amenta, 1993).

On the basis of our data alone we cannot speculate about the possible functional significance of Ca^{2+} channels of the L-type in human peripheral blood lymphocytes and whether lymphocytes express other types of Ca^{2+} channels. Further work is in progress to clarify the possible influence of Ca^{2+} channel blockers in modulating immune function, and if the binding site characterised in this study may be relevant as far as the therapeutic effect of Ca^{2+} channel antagonists is concerned.

Acknowledgements

The present study was supported in part by grants of 'La Sapienza' University (Rome, Italy) and of the Italian National Research Council (C.N.R.).

References

Bishopric, N.H., H.J. Cohen and R.J. Lefkowitz, 1980, Beta-adrenergic receptors in lymphocyte sub populations, J. Allergy Clin. Immunol. 65, 29.

Bolger, G.T., P. Gengo, R. Klockowski, E. Luchowski, H. Siegel, A. Janis and A.M. Triggle, 1983, Characterization of binding of the Ca^{2+} channel antagonist [^{3}H]nitrendipine, to guinea-pig ileal smooth muscle, J. Pharmacol. Exp. Ther. 225, 291.

Cheng, Y.C. and W.H. Prusoff, 1973, Relationship between the inhibition constant (K_i) and the concentration of inhibitor which causes 50% inhibition (IC_{50}) of an enzyme reaction, Biochem. Pharmacol. 22, 3099.

Chitwood, K.K. and K.L. Heim-Duthoy, 1993, Immunosuppressive properties of calcium channel blockers, Pharmacotherapy 13, 447.

Cortes, R., P. Supavilai, M. Karobath and J.M. Palacios, 1983, The effects of lesions in the rat hippocampus suggest the association of calcium channel blocker binding sites with specific neuronal population, Neurosci. Lett. 42, 249.

Cortes, R.P. Supavilai, M. Karobath and Palacios, 1984, Calcium channel antagonist binding sites in the rat brain: quantitative autoradiographic mapping using the 1,4-dihydropydines [^{3}H]PN 200-110 and [^{3}H]PY 108-068, J. Neural Transm. 60, 169.

Dugas, B., A. Vazquez, J.-F. Delfraissy, J.-P. Gérard, M.-T. Rannou and P. Galanaud, 1986, Human B cell activation: selective sensitivity of early stages to human calcium channel-blocking drugs, Eur. J. Immunol. 16, 162.

Ferrante, F. and F. Amenta, 1993, Autoradiographic localization of [^{3}H]nicardipine binding sites in the human renal artery, Eur. J. Pharmacol. 240, 229.

Ferry, D.R., A. Goll and H. Glossmann, 1983, Differential labelling of putative skeletal muscle calcium channels by [^{3}H]-nifedipine, [^{3}H]-nitrendipine, [^{3}H]-nimodipine and [^{3}H]-PN 200-110, Naunyn-Schmied. Arch. Pharmacol. 323, 276.

Fleckenstein, A., 1983, Calcium Antagonism in Heart and Smooth Muscle (J. Wiley and Sons, New York).

Freedman, M.H, N.R. Khan, B.J. Trew-Marshall, C.G. Cupples and B. Mely-Goubert, 1981, Early biochemical events in lymphocyte activation, Cell. Immunol. 58, 134.

Glossmann, H. and D.R. Ferry, 1985, Assay for calcium channels, Methods Enzymol. 109, 513.

Glossmann, H., D.R. Ferry, F. Lubbecke, R. Mewes and F. Hofmann, 1982, Calcium channels: direct identification with radioligand binding studies, Trends Pharmacol. Sci. 3, 431.

Godfraind, T., N. Morel and M. Wibo, 1988, Tissue specificity of dihydropyridine-type Ca^{2+} channel antagonist in isolated human tissues, Trends Pharmacol. Sci. 9, 37.

Kay, J.E., 1971, Interactions of lymphocytes and phytoemoagglutinin inhibition by chelating agents, Exp. Cell Res. 68, 11.

Kokobun, S., B. Prod'hom, C. Becker, H. Porzig and H. Reuter, 1986, Studies on Ca^{2+} channels in intact cardiac cells: voltage-dependent effects and co-operative interaction of dihydropyridine enantiomers, Mol. Pharmacol. 30, 571.

Kunert-Radek, J., H. Stepien, K. Lyson and M. Pawlikowski, 1990, Effects of calcium channel modulators on the proliferation of mouse spleen lymphocytes *in vitro*, Agents Actions 29, 254.

Latifpour, J., Y. Masaki and R.M. Weiss, 1992, Evidence for the presence of regional differences in the calcium antagonist receptors in lower urinary tract smooth muscle, Naunyn-Schmied. Arch. Pharmacol. 345, 679.

Lewis, R.S. and M.D. Cahalan, 1990, Ion channels signal transduction in lymphocytes, Annu. Rev. Physiol. 52,415.

Maslinski, W.,1989, Cholinergic receptors of lymphocytes, Brain Behav. Immunol. 3, 1.

Morel, N. and T. Godfraind, 1988, Selective modulation by membrane potential of the interaction of some calcium entry blockers with calcium channels in rat mesenteric artery, Br. J. Pharmacol. 95, 252.

Morgano, A., I. Pierri, R. Stagnaro, M. Setti, F. Puppo and F. Indiveri, 1990, Decreased lymphocyte blastogenesis, IL2 production and NK activity following nifedipine administration to healthy humans, Eur. J. Clin. Pharmacol. 39, 545.

Murphy, K.M.M. and S.H. Snyder, 1982, Calcium antagonist receptor binding sites labelled with [^{3}H]nitrendipine, Eur. J. Pharmacol. 77, 770.

Nagai, Y., S. Ueno, Y. Saeki, F. Soga and T. Yanagihara, 1993, Expression of the D_3 dopamine receptor gene and a novel variant transcript generated by alternative splicing in human peripheral blood lymphocytes, Biochem. Biophys. Res. Commun. 194, 386.

Nayler, W.G., 1988, Calcium Antagonists (Academic Press, London).

Resch, K., D. Bouillon and D. Gemsa, 1978, The activation of lymphocytes by the ionophore A23187, J. Immunol. 120, 1514.

Ricci, A. and F. Amenta, 1994, Dopamine D_5 receptors in human peripheral blood lymphocytes: a radioligand binding study, J. Neuroimmunol. 53, 1.

Schwartz, A. and D.J. Triggle, 1984, Cellular action of calcium channel blocking drugs, Annu. Rev. Med. 35, 325.

Scriabine, A., T. Schuurman and J. Traber, 1989, Pharmacological basis for the use of nimodipine in central nervous system disorders, FASEB J. 3,1799.

Slish, D.F., D.B. Engle, G. Varani, I. Lotan, D. Singer, N. Dascal and A. Schwartz, 1989, Evidence for the existence of a cardiac specific isoform of the a_1 subunit of the voltage dependent calcium channel, FEBS Lett. 250, 509.

Spedding, M. and R. Paoletti, 1992, Classification of calcium channels and the sites of action of drug modifying channel function, Pharmacol. Rev. 44, 363.

Takahashi, N., Y. Nagai, S. Ueno, Y. Seaki and T. Yanagihara, 1992, Human peripheral blood lymphocytes express D5 dopamine receptor gene and transcribe the two pseudogenes, FEBS Lett. 314, 23.

Triggle, D.J., 1991, Sites, mechanisms of action and differentiation of calcium channel antagonists, Am. J. Hypertension 4, 225.

Weir, M.R., 1991, Therapeutic benefits of calcium channel blockers in cyclosporine-treated organ transplant recipients: blood pressure control and immunosuppression, Am. J. Med. 90 (Suppl. 5A), 32S.

Weir, M.R., R. Peppler, D. Gomolka and B.S. Handwerger, 1992, Evidence that the antiproliferative effect of verapamil on afferent and efferent immune responses is independent of calcium channel inhibition, Transplantation 54, 681.

Whitney, R.B. and R.M. Sutherland, 1972, Enhanced uptake of calcium by transforming lymphocytes, Cell. Immunol. 5, 137.

Wibo, M., L. Deroth and D. Godfraind, 1988, Pharmacologic relevance of dihydropyridine binding sites in membranes from rat aorta: kinetic and equilibrium studies, Circ. Res. 62, 91.

Wybran, J., 1986, Enkephalins as molecules of lymphocytes activation and modifiers of the biological response, in: Enkephalins and Endorphins, eds. N.P. Plotnikoff, R.E. Faith, A.J. Murgo and R.A. Good (Plenum, New York) p. 253.

Yamada S., Y. Harada and K. Nakamura, 1988, Characterization of calcium channel antagonist binding sites labelled by [^{3}H]nitrendipine in porcine coronary and aorta, Eur. J. Pharmacol. 154, 203.