FEBS Letters 364 (1995) 120–124 FEBS 15438

Down-regulation of β-adrenergic receptors induced by mitogen activation of intracellular signaling events in lymphocytes

C.A. Cazaux, L. Sterin-Borda, G. Gorelik, G.A. Cremaschi*

Centro de Estudios Farmacológicos y Botánicos (CEFYBO-CONICET), Serrano 669, 1414 Buenos Aires, Argentina

Received 20 March 1995

Abstract The expression of β-adrenergic receptors on murine lymphocytes stimulated with concanavalin A was studied. A decrease in β-adrenoceptor number on T lymphocytes and a diminished response to specific agonist stimulation at the peak of proliferation was found. The blockade of cell proliferation by tyrosine kinases or protein kinase C inhibitors reversed the decrease in β-adrenoceptor number. PMA plus ionophore or interleukin-2 but not PMA alone were able to induce β-adrenoceptor down-regulation accompanying cellular proliferation. These results showed that the intracellular signals triggered during lymphocyte activation are involved in β-adrenoceptor down-regulation and it would represent the loss of a mechanism that exerts negative neuroimmune control of cellular proliferation.

Key words: β-Adrenoceptor; Proliferation; Tyrosine kinase; Protein kinase; Intracellular signaling

1. Introduction

The activation of T cells by specific antigen, mitogen or monoclonal antibodies leading to T lymphocyte proliferation and differentiation is mediated by a complex series of intracellular signaling events. The signal transduction pathway is initiated by the obligatory activation of tyrosine protein kinases (TPK) [1,2]. This results in activation of phospholipase C (PLC) which hydrolyzes membrane inositol phospholipids and gives rise to two 2nd messengers, namely inositol triphosphate ($InsP_3$) and diacylglycerol (DAG). $InsP_3$ increases the concentration of intracellular calcium and DAG activates distinct isoforms of protein kinase C (PKC) which then phosphorylate and regulate various downstream targets [1,3]. The distinct effects of these two 2nd messengers can be partially mimicked by Ca^{2+} ionophores, which increase the intracellular free Ca^{2+} concentration and phorbol esters (for example, PMA) which activate PKC [4]. PMA binds to and activates PKC at physiologic Ca^{2+} concentrations [5] inducing proliferation of human peripheral blood lymphocytes [6].

Evidence has been accumulating over the past several years indicating the participation of the autonomic sympathetic system in the modulation of lymphocyte activity. In fact, several studies have identified and characterized β-adrenergic receptor (R) on lymphocytes [7] and showed that their activation could exert a control of lymphocyte proliferation by increasing the intracellular levels of cyclic AMP (cAMP) [8,9]. Thus we have previously described the absence of functional β-adrenoceptors in several hyperproliferative lymphoma cell lines [10,11]. Furthermore, it was described that β-adrenergic receptor sites are diminished in activated B lymphocytes during the course of alloimmunization [12].

The present study was undertaken to analyze the role of the sympathetic nerve system upon lymphocyte proliferation. We here shown that β-adrenergic expression and function were diminished on T lymphocytes stimulated with the mitogenic lectin concanavalin A (Con A) and that the intracellular pathways triggered during lymphocytes activation are involved in β-adrenergic R down-regulation.

2. Materials and methods

2.1. Cell suspensions and culture conditions

Lymphoid cell suspensions from BALB/c inbred mice lymph nodes, as well as nylon wool purified T cells, were prepared aseptically as described before [13,14]. Cells were cultured at a concentration of 1×10^6 cell/ml, in RPMI 1640 medium (Gibco Co.) supplemented with 10% heat-inactivated fetal calf serum, 2 mM glutamine and antibiotics, alone or in the presence of one of the stimulators of proliferation: Con A (4 μg/ml; Sigma Chemical Co.) or the phorbol ester, phorbol myristate acetate (PMA, 2×10^{-8} M; Sigma) alone, together with ionophore A23187 (2×10^{-6} M; Sigma) or with 12.5 IU/ml of human recombinant IL-2 (rIL-2) (Electro Nucleonics Inc.). For microcultures they were settled at a final volume of 0.2 ml in 96-well flat-bottom microtiter plates (Corning, NY) and for macrocultures they were kept in T-25 culture flasks (Corning). Cells were cultured for different times as indicated. Where indicated, inhibitors or anti-Tac monoclonal antibody (anti-IL-2 R, 1 : 10; Becton Dickinson) were added when the culture was initiated.

2.2. Proliferation assays

They were performed in microcultures and in 0.2 ml aliquots of macrocultures separated on the indicated times and pulsed for 6 h with [^{3}H]thymidine ([^{3}H]TdR (INC, Irvine, CA, USA); 15 Ci/mmol) as described [11]. Results are expressed as stimulation index (S.I.) calculated as the rate between dpm values in experimental cultures and dpm from control values obtained with unstimulated cells. For experimental cultures performed in the presence of inhibitors, % inhibition (% inh.) was calculated as:

$$\%\text{inh.} = 1 - \frac{\text{dpm Con A stimulated cells + inhibitor} - \text{dpm control}}{\text{dpm Con A stimulated cells} - \text{dpm control}} \times 100.$$

2.3. [^{125}I]Cyanopindolol ([^{125}I]CYP) binding to intact cells

$2–3 \times 10^6$ cells/tube were incubated with [^{125}I]CYP (New England Nuclear; 2200 Ci/mmol) solutions, 1–300 pM radioligand concentration in 50 mM phosphate buffer, made isotonic with NaCl, 10 mM $MgCl_2$ and 10^{-4} M phentolamine. After 30 min incubation at 30°C samples were filtered through Whatman GF/C filters as indicated before [10] and filters were counted in a Wallac counter. Total binding curves were analyzed by computer program LIGAND as already described [10,11]. The non-specific binding parameter fitted by LIGAND from the total binding curves did not differ from those determined experimentally using 1 μM L-propranolol.

2.4. Cyclic-AMP production

$8–10 \times 10^6$ cells/ml were incubated in RPMI 1640 for the indicated

*Corresponding author. Fax: (54) (1) 856-2751.

SSDI 0014-5793(95)00366-5

times and concentrations, with the β-agonist (−)-isoproterenol (ISO) or with prostaglandin E_1 (PGE_1). Samples were prepared and cyclic nucleotides were measured as described before [10,11].

2.5. Drugs

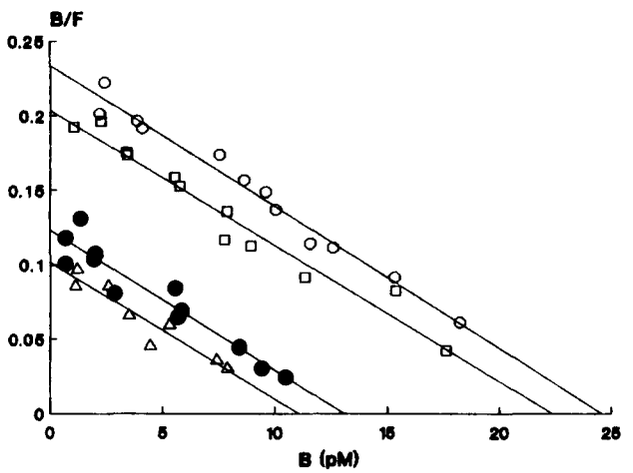
The following drugs were used in culture at the final concentrations indicated in results. The protein kinase inhibitors 1-(5-isoquinolinesulfonyl)-2-methylpiperazine dihydrochloride (H-7) and *N*-(2-guanidinoethyl)-5-isoquinoline sulfonamide hydrochloride (HA 1004), and the TPK inhibitor genistein [15], were obtained from Seikagaku Kogyo (Tokyo, Japan) and from Sigma, respectively.

2.6. Statistical analysis

The Student's *t*-test for unpaired values was used to determine the levels of significance. When multiple comparison was necessary after analysis of variance, the Student-Newman-Keuls test was applied. Differences between means were considered significant if $P \leq 0.05$.

3. Results and discussion

For analyzing the expression of β-adrenergic R on Con A-stimulated T lymphocytes macrocultures of lymph node cells were prepared. Cells in macrocultures displayed the same kinetic of proliferation as that expected for Con A stimulated microcultures with the peak of proliferation at the third day of culture that is coincident with a significant decrease in β-adrenoceptor number (Fig. 1). This was not due to culture conditions, as unstimulated cells kept in culture medium for 3 days showed no reduction in β-adrenergic R number (Fig. 1) and it was similar to the decrease obtained with T lymphocytes enriched populations stimulated for 3 days with Con A (B_{max}: 401 ± 12 sites/cell). It is worth noting that at day 1 of culture, no modifications were observed in the maximum number of binding sites with respect to the R found in normal T lymphocytes (697 ± 27 sites/cell) and that the reduction persisted after 5 days of culture, when proliferating rate was diminished (Fig. 1). Furthermore, we found a significantly lower response in isoproterenol (ISO) stimulated cAMP production on Con A treated cells from 3 days of culture as compared to normal lymphocytes (data not shown) or cells from 1 day of culture with normal number of β-adrenoceptors, reflecting the reduction of β-adrenergic sites (Fig. 2). However, similar responses were found in both cases when stimulation was induced by prostaglandin E_1 (PGE_1), another hormone that also activates the adenylate cyclase system. These data indicate that the still available receptor sites are functional, differently to what occurs with hyperproliferative cell lines with reduced number of uncoupled β-adrenergic receptors [10]. This phenomenon would probably indicate the loss of a negative control of lymphocyte proliferation as it was previously demonstrated that proliferation of spleen lymphocytes was inhibited by increasing intracellular cAMP levels [16]. To study if the intracellular events that lead to T lymphocyte proliferation were involved in this phenomenon the effect of different PK and TPK blockers on proliferation and on β-adrenergic receptor expression was compared. Fig. 3 shows that Con A stimulation was inhibited in a dose-related manner by the TPK inhibitor genistein and a full inhibition was achieved with 20–30 μg/ml of the inhibitor. Under these conditions β-adrenergic sites were increased although to a lower extent than in control normal lymphocytes values (Table 1). It is worth noting that these doses of the inhibitor have no effect on cultured cells viability (% of



Cells stimulated with		Days of Culture	Binding of ^{125}I-CYP Kd ± S.D. (pM)	Bmax ± S.D. (sites/cell)	S.I.
Con A	○--○	1	94.4 ± 5.4	859 ± 42	2.0 ± 0.5
	●--●	3	81.4 ± 6.8	371 ± 48*	100.3 ± 15.0
	△--△	5	76.7 ± 7.6	341 ± 20*	20.8 ± 1.0
Control	□--□	3	82.3 ± 6.1	772 ± 69	-

Fig. 1. [^{125}I]CYP binding to cultured lymph node cells. Cells kept in culture for the indicated days, stimulated or not with Con A (4 μg/ml), were assayed for [^{125}I]CYP binding as indicated in section 2. Scatchard plots are representative of 4 experiments. Mean values of K_d and B_{max} ± S.D. are shown in the Table below the figure together with the stimulation index (S.I.) obtained by [^{3}H]TdR incorporation for comparison. *Differ from control value of unstimulated T lymphocytes with $P < 0.001$.

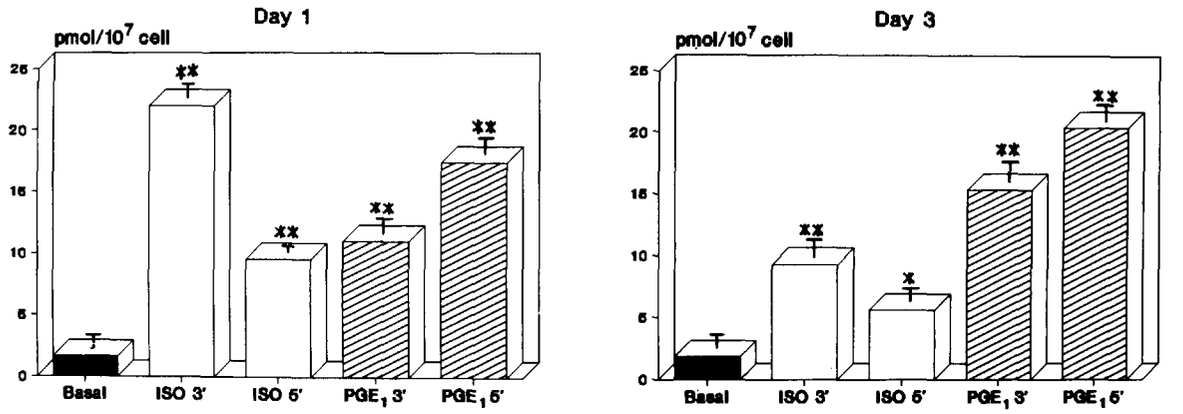


Fig. 2. Measurement of cAMP levels in Con A stimulated lymph node cells. Cells were cultured for 1 or 3 days and cAMP was measured on cells alone (Basal; ■) or stimulated for 3 or 5 min with (−)-isoproterenol (ISO; 10 μM) (□) or with prostaglandin E_1 (PGE_1; 10 μM) (▨). Results are the mean of 3 experiments performed in triplicate. Differ significantly from the corresponding basal values with: $^*P < 0.05$, $^{**}P < 0.01$. ISO values from day 1 of culture are significantly higher than those obtained at day 3, with: (a) $P < 0.01$ for ISO 3′ and $P < 0.05$ for ISO 5′. Non-statistical differences were found between peak values of PGE_1-stimulated cAMP values from day 1 and 3.

viable cells with 30 μg/ml was ≥ 70%). An explanation for the incomplete recovery of β-adrenoceptors would be found in the fact that this dose of genistein was demonstrated to inhibit only partially the total TPK activity in T lymphocytes [1].

Additionally the possibility that the following steps of lymphocyte activation cascade would be involved, was investigated by using PK inhibitors, H-7 and HA1004. H-7 and HA1004 inhibit PKC and cyclic nucleotide PK, being H-7 more efficient in inhibiting PKC than HA1004 [19]. H-7 but not HA1004 (up to 20 μM, dose that not affect PKC) inhibited Con A stimulated proliferation in a dose-related manner (Fig. 3).

Moreover, as is shown in Table 1 the expression of β-adrenoceptors was unaffected and was kept as in control values when H-7, but not HA1004, was used at 5 μM, a dose that inhibits PKC [17], but only inhibit blast transformation near to 50% (Fig. 3). These results indicate that PKC activation leading to proliferation is involved in β-adrenoceptor down-regulation which is not a mere consequence of blast transformation during proliferation.

To investigate in depth PKC involvement in the down-regulation of β-adrenoceptors, we analyzed if its activation would lead to reduction in β-adrenergic receptor number. As sustained activation of PKC is essential for subsequent responses such as cell proliferation and differentiation [18], and as PMA causes sustained activation of PKC [18,19], its effects upon lymph node cell proliferation and β-adrenergic R expression were studied. Studies were not performed on purified T cells since it has been shown [20] that mitogenic effects of PMA on resting T cells depends on accessory cells and are not observed when purified T cells are used. PMA behaves as a weak mitogen upon lymph node cell proliferation at third day of culture (SI in Fig. 4) as was previously shown in human peripheral blood lymphocytes [20]. However, no reduction of β-adrenergic sites was found at any time of cell culture (Fig. 4) and even a slight increase in β-adrenergic R sites was found at 3rd day of culture.

As it was shown that Ca^{2+} ionophore acts synergistically with phorbol esters to stimulate DNA synthesis and in the activation of some isoenzymes of PKC, namely PKCα and PKCβ in human T cells [21], we studied whether A23187 would act synergistically with PMA to induce β-adrenoceptor down-regulation. We found that cells cultured for 3 days with A23187 alone displayed a slight decrease in β-adrenoceptor number (613 ± 36 sites/cell), that represents not more than 12% reduction with respect to normal T cell controls, and no proliferative activity

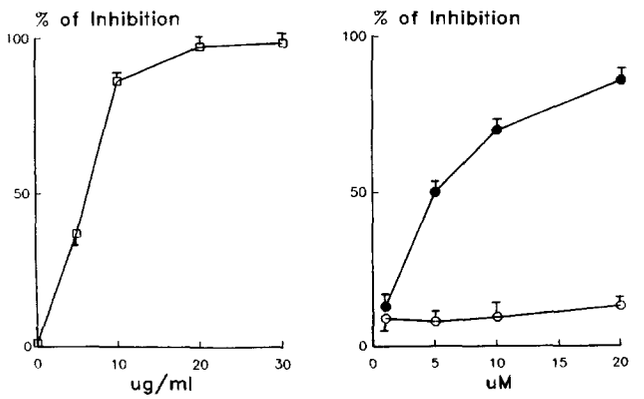
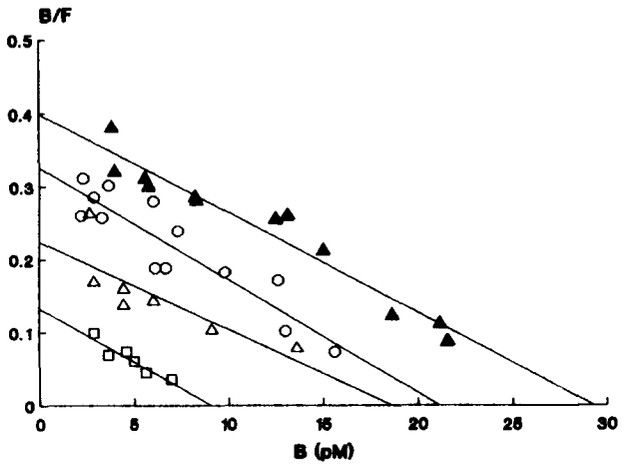


Fig. 3. Effect of TPK and PK inhibitors on Con A-stimulated proliferation. Dose–response curves showing the effects of the TPK blocker, genistein (□), and the PK inhibitors: H-7 (●) or HA 1004 (○). Drugs were added at time zero (when initiation of culture) and cells were harvested at the third day after a 6-h long pulse of [^{3}H]TdR. Results are the mean % of inhibition of at least 3 experiments ± S.D.

Table 1
Inhibition of Con A-induced decrease in β-adrenoceptor number by PKC and TPK blockers

Treatment[a]	[^{125}I]CYP binding[b]	
	K_d ± S.D. (pM)	B_{max} (sites/cell)
Con A	81.4 ± 6.8	371 ± 48
Con A + genistein (30 μg/ml)	84.2 ± 7.4	616 ± 37*
Con A + H-7 (5 μg/ml)	79.6 ± 5.3	743 ± 12*
Con A + HA 1004 (5 μg/ml)	82.7 ± 6.9	401 ± 16

[a]Cells were cultured with Con A alone or in the presence of the indicated TPK or PK blockers. After 3 days of culture, cells were washed three times and measurement binding of [^{125}I]CYP was performed.
[b]K_d and B_{max} values were calculated by LIGAND analysis as indicated.
*Differ significantly from Con A values with $P < 0.001$.



Cells stimulated with		Days of Culture	Binding of ^{125}I-CYP Kd ± S.D. (pM)	Bmax ± S.D. (sites/cell)	S.I.
	○--○	1	73.0 ± 7.1	843 ± 86	1.5 ± 0.5
PMA	▲--▲	3	78.5 ± 7.5	1023 ± 96	18.8 ± 8.2
	△--△	5	85.8 ± 7.6	762 ± 59	8.1 ± 1.6
PMA + A23187	□--□	3	73.1 ± 6.2	349 ± 33*	25.7 ± 6.7

Fig. 4. Binding of the β radioligand [^{125}I]CYP to cells stimulated with PMA or with PMA plus ionophore. Cells were cultured for the indicated days with 2 nM PMA or for 3 days with 2 nM PMA plus 2 μM A23187 and β-adrenoceptor expression was checked by [^{125}I]CYP binding as referred to before. The K_d, B_{max} and S.I. values listed below were obtained as indicated in Fig. 1. *Differ significantly from control or PMA alone with $P < 0.001$.

was observed (SI = 1.4). However, when lymph node cells were incubated with both Ca^{2+} ionophore and PMA, a higher proliferative response and a significant reduction in β-adrenoceptor sites were achieved (Fig. 4), indicating that A23187 acts synergistically with PMA to induce β-adrenergic R down-regulation. Furthermore, the Ca^{2+} blocker verapamil (10^{-5} M) abrogated PMA plus ionophore effects both on proliferation and on β-adrenergic R expression (data not shown). As Ca^{2+} uptake seems to play a major role in the events leading to IL-2 production [22,23] and phorbol ester treatment is sufficient for induction of the IL-2 R, but synthesis and secretion of IL-2 as well as IL-2 dependent T cell proliferation require the additional signal provided by Ca^{2+} ionophores [24] the possible participation of IL-2 in diminishing β-adrenoceptor expression was analyzed on lymphocyte cultures stimulated with PMA plus rIL-2. As is shown in Table 2, rIL-2 addition (12.5 IU/ml) to PMA stimulated T cells synergize the proliferative action of PMA ($S.I._{PMA} = 19$ vs. $S.I._{PMA+rIL\text{-}2} = 30$) and lead to β-adrenoceptor down-regulation at 3rd day of culture. Furthermore, as it was demonstrated that blockade of the IL-2 R by anti-Tac antibody inhibited lymphocyte proliferation induced by Con A stimulation to about 50–88% [25], we analyzed anti-Tac effects on Con A induced proliferation and β-adrenergic R down-regulation. Table 2 shows that anti-Tac inhibits Con A proliferative activity to 42% and reverted the decrease of β-adrenergic R sites almost to basal values.

As a consequence of T lymphocyte activation T cells undergoes morphologic changes (blastogenesis) at about 12 h, divides by 24–48 h and then differentiates as genes are sequentially activated for several days [26]. The observed effects upon β-adrenergic receptor expression were not due just to blast transformation as cells from 1 day of culture showed no reduction of β-adrenoceptor sites. Loss of R might be a result of R internalization and degradation as a consequence of mitogen induced kinase activation which would lead to β-adrenergic heterologous desensitization. Although this possibility cannot be ruled out, it would be difficult to explain the normal

Table 2
Participation of IL-2 in β-adrenergic down-regulation

Treatment[a]	[^{3}H]TdR incorporation[b] (dpm)	[^{125}I]CYP binding[c] B_{max} (sites/cell)
Basal	1,256 ± 89	831 ± 37
PMA	23,822 ± 612	1,131 ± 94
PMA + IL-2	37,985 ± 1,760	544 ± 45*
Con A	110,371 ± 1,935	402 ± 42**
Con A + anti-Tac	64,015 ± 1,129	726 ± 33

[a]Cells were cultured for 3 days either alone or in the presence of 2 nM PMA; same amount of PMA plus rIL-2 (12.5 U/ml). 4 μg/ml Con A or also in the presence of anti-Tac (1:10 dilution).
[b]Mean dpm values ± S.D. for [^{3}H]TdR incorporated at the third day of culture for 3 experiments performed in triplicate.
[c]B_{max} values were calculated as indicated in Table 1.
*Differ significantly from PMA values with $P < 0.01$.
**Differ from basal values with $P < 0.025$.

response to β-agonist stimulation of cells culture with the mitogen for 1 day, as it was previously shown that heterologous desensitization is associated with a rapid functional uncoupling of the receptor from the adenylate cyclase system in the absence of R sequestration or down-regulation [27]. Another possibility is that the observed effects are a consequence of the cascade of intracellular biochemical events that transduce the signal across the outer membrane into the cell nucleus triggering a genetically predetermined program which would include the down-regulation of β-adrenoceptors.

In support of this blockade of TPK activity, the first step of T lymphocyte activation induced signal transduction pathway inhibits β-adrenoceptor site reduction at third day of culture. Modulation of β-adrenergic receptor signal transduction by TPK was described in fibroblasts [28] and the possibility that this modulation would indirectly involve PKC participation was not discarded [28].

In fact, the direct activation of PKC with PMA has no effect on β-adrenoceptor expression, but PKC synergistic activation by phorbol ester plus ionophore mimicked the Con A induced reduction in β-adrenoceptor expression and PKC blockade impaired β-adrenoceptor down-regulation. Both mitogenic lectin and PMA plus ionophore induced IL-2 gene activation [26]. However, PMA and other tumor promoters induce proliferation via an IL-2-independent mechanism [20,29]. So the participation of IL-2 in β-adrenoceptor down-regulation was analyzed. IL-2 R blockade on Con A-stimulated lymphocytes impaired β-adrenoceptor down-regulation, while the addition of IL-2 together with PMA to lymphocyte cultures also induced the decrease of β-adrenergic binding sites. In support of these results, inhibition of TPK by genistein [30] and of PKC by H-7 [17] inhibit IL-2 secretion and IL-2 R expression in a dose-related manner and both inhibitors reverted Con A induced β-adrenergic R down-regulation.

Evidence of β-adrenoceptor down-regulation as a change in the synthesis of R at the gene level was demonstrated by Kiely et al. [31] in C_6 glioma cells, where glucocorticoids induced R down-regulation suppressing transcription of gene encoding for the R.

An increased degradation of β-adrenoceptors would also be genetically determined by the cascade of intracellular signals triggered during proliferation. Evidence is now mounting that polypeptide ligands and their membrane R may have an important additional signaling role within the cell nucleus [32].

The genetic signals triggered by the biochemical cascade of intracellular messengers that would be involved in this down-regulation are now under study. This phenomenon would implicate the loss of a mechanism that exerts negative neuroimmune control of cellular proliferation.

Acknowledgements: The authors wish to thank Drs. E.S. Borda and A.M. Genaro for their helpful discussion of the manuscript and Mrs. E. Vannucchi for her excellent technical assistance. This work was supported by Grants 3025100/93 from CONICET.

References

[1] Mustelin, T., Coggeshall, K.M., Isakov, N. and Altman, A. (1990) Science 247, 1584–1587.
[2] Harnett, M. and Rigley, K. (1992) Immunol. Today 13, 482–486.
[3] Houslay, M.D. (1991) Eur. J. Biochem. 195, 9–27.
[4] Truneh, A., Albert, F., Golstein, P. and Schmitt-Verhulst, A.M. (1985) Nature 313, 318–320.
[5] Castagna, M., Takai, Y., Kaibuchi, K., Sano, K., Kikkaura, V. and Nishizuka, Y. (1982) J. Biol. Chem. 257, 7847–7851.
[6] Kaibuchi, K., Takai, Y. and Nishizuka, Y. (1985) J. Biol. Chem. 260, 1366–1369.
[7] Plaut, M. (1987) Annu. Rev. Immunol. 5, 621–669.
[8] Coffey, R.G. and Hadden, J.W. (1985) Fed. Proc. 44, 112–117.
[9] Roszman, T.L. and Brooks, W.H. (1988) Prog. Allergy 43, 140–159.
[10] Cremaschi, G.A., Fisher, P. and Boege, F. (1991) Immunopharmacology 22, 195–206.
[11] Cremaschi, G.A., Miguel, S., Cazaux, C. and Sterin-Borda, L. (1994) Cell. Signalling (in press).
[12] Genaro, A.M. and Borda, E. (1989) Immunopharmacology 18, 63–70.
[13] Cremaschi, G.A., Genaro, A.M. and Sterin-Borda, L. (1989) Mol. Immunol. 26, 601–609.
[14] Genaro, A.M., Borda, E., Cremaschi, G.A., Sterin-Borda, L. and Braun, M. (1986) Immunopharmacology 11, 129–140.
[15] Enright, W.J. and Booth, P. (1991) Focus 13, 79–83.
[16] Zhou, P., Gorzynski, T., Dowjat, W.K., Rabin, R. and Zaleski, M.B. (1989) Exp. Cell. Biol. 57, 346–357.
[17] Atluru, D., Polam, S., Atluru, S. and Woloschak, G.E. (1990) Cell. Immunol. 129, 310–320.
[18] Nishizuka, Y. (1992) Science 258, 607–613.
[19] Nishizuka, Y. (1989) J. Am. Med. Assoc. 262, 1826–1833.
[20] Isakov, N., Galron, D., Mustelin, T., Pettit, G.R. and Altman, A. (1993) J. Immunol. 150, 1195–1204.
[21] Isakov, N., Mally, M.I. and Altman, A. (1991) Mol. Immunol. 29, 927–933.
[22] Mills, G.B., Cheung, R.K., Goinstein, S. and Gelfand, E.W. (1985) J. Immunol. 134, 1640.
[23] Gelfand, E.W. (1991) Clinical Immunol. Immunopathol. 61, 51–59.
[24] Truneh, A., Albert, F., Golstein, P. and Schmitt-Verhulst, A.M. (1985) Nature 313, 318.
[25] Depper, J.M., Leonard, W.J., Robb, R.J., Waldmann, T.A. and Greene, W.C. (1983) J. Immunol. 131, 690–696.
[26] Crabtree, G.R. (1989) Science 243, 355–361.
[27] Sibley, D.R., Benovic, J.L., Caron, M.G. and Lefkowitz, R.J. (1988) Endocr. Rev. 9, 38–56.
[28] Bushman, W.A., Wilson, L.K., Luttrell, D.K., Moyers, J.S. and Parsons, S.J. (1990) Proc. Natl. Acad. Sci. USA 87, 7462–7466.
[29] Isakov, N., Bleackley, R.C., Shaw, J. and Altman, A. (1985) J. Immunol. 135, 2343–2347.
[30] Trevillyan, J.M., Lu, Y., Atluru, D., Phillips, C.A. and Bjorndahl, J.M. (1990) J. Immunol. 145, 3223–3230.
[31] Kiely, J., Hadcock, J.R., Bahouth, S.W. and Malbon, C.C. (1994) Biochem. J. 302, 397–403.
[32] Jans, D.A. (1994) FASEB J. 8, 841–847.