FEBS Letters 358 (1995) 158–160 FEBS 15029

Rapamycin, FK506 and cyclosporin A inhibit human prolactin gene expression

Stefaan Wera, Alexandra Belayew, Joseph A. Martial*

Laboratoire de Biologie Moléculaire et de Génie Génétique, Université de Liège, Institut de Chimie B6, B-4000 Sart-Tilman, Belgium

Received 28 November 1994; revised version received 13 December 1994

Abstract In this work we demonstrate that transcription of the human prolactin gene is inhibited by the immunosuppressants FK506 (IC_{50} = 25 nM), cyclosporin A (IC_{50} = 190 nM) and rapamycin (IC_{50} = 25 nM). Whereas the effect of FK506 and cyclosporin A is specific for prolactin gene transcription, rapamycin has a more general effect on transcription and/or translation in pituitary cells. In view of recent work demonstrating the immunoactivating role of prolactin, these results suggest that inhibition of prolactin gene expression in the pituitary may contribute to the mechanism of action of immunosuppressants.

Key words: Prolactin; FK506; Rapamycin; Cyclosporin A

1. Introduction

Recently the mechanism of action of the widely used immunosuppressant drug cyclosporin A was elucidated [1–3]. Cyclosporin A enters T-lymphocytes where it associates with an intracellular receptor termed cyclophilin. The complex between cyclosporin A and cyclophilin, but not the individual components, is a potent inhibitor of the Ca^{2+}-dependent protein phosphatase calcineurin [1,2]. Inhibition of calcineurin leads to aberrant phosphorylation of transcription factors and this prevents transcription of the interleukin-2 gene, an essential step in T-lymphocyte activation. The novel immunosuppressant FK506 acts in a very similar way since the complex between FK506 and its intracellular receptor (FKBP12) is a potent inhibitor of calcineurin [1–3].

The immunosuppressant rapamycin also associates with FKBP12, but this complex does not affect calcineurin activity. The rapamycin–FKBP12 complex inhibits the signaling pathway distal of the interleukin-2 receptor, most likely via binding to a protein termed FRAP [4].

Prolactin is an important regulator of the immune system (reviewed in [5]) and appears to have a non-specific immunoactivating function. Prolactin is mitogenic for T-lymphocytes [6]. Administration of antisera to prolactin affects the development of T-lymphocytes in the thymus and the spleen [7]. T-lymphocytes express both prolactin and prolactin receptors [8]. In view of this immunoregulatory role of prolactin we decided to investigate whether immunosuppressants could influence pituitary prolactin gene expression.

*Corresponding author. Fax: (32) (41) 562 968.

Abbreviations: CAT, choramphenicol acetyltransferase; hPRL, human prolactin; TK, thymidine kinase.

2. Experimental

2.1. Materials

FK506 and rapamycin were kind gifts of Dr. K. Murato (Fujisawa GmbH) and Dr. L. Faucette (Smith-Kline Beecham), respectively. Cyclosporin A was from Sandoz. Plasmids containing the CAT reporter gene linked either to the hPRL promoter or the TK promoter are described in [9].

2.2. Cell culture and transfection

GH_3 rat pituitary tumor cells were cultured and transfected by electroporation as described [9], except that an Easyject apparatus (Eurogentec/Equibio, Seraing, Belgium) was used. Briefly, 10^7 cells were gently mixed with 20 μg plamid DNA and electroporated with a single pulse of 1500 μF and 270 V. Cells were incubated for 16 h, then harvested and processed for CAT assay as described [9,10].

Toxicity of the various drugs was tested by counting Trypan blue-excluding cells after an overnight incubation of 0.25×10^6 cells with the indicated concentrations of the drug. Results are expressed as means ± S.E.M. Illustrated results are the mean of 2 experiments each performed in triplicate.

3. Results

3.1. Immunosuppressants inhibit human prolactin gene expression

Human prolactin (hPRL) gene expression in the pituitary is tightly controlled via transcription factors that bind to the *cis*-acting elements present in the promoter region of the gene. We showed previously [9,11] that a CAT reporter gene fused to the promoter of the human prolactin gene is a reliable model to study prolactin gene expression. In order to study the effect of immunosuppressants on transcription of the hPRL gene, we transfected rat pituitary tumor cells (GH_3) with a construct containing 3500 bp of the hPRL promoter linked to a CAT reporter gene. In the experiment shown in Fig. 1A, increasing concentrations of FK506 progressively inhibited hPRL3500-CAT transient expression up to 75% (IC_{50} = 25 nM). Expression of a control TKCAT vector, carrying the neutral promoter of the Herpes virus thymidine kinase gene linked to a CAT reporter gene, was not affected by FK506. Addition of cyclosporin A (Fig. 1B) caused a less pronounced inhibition of hPRL3500CAT expression (IC_{50} = 190 nM) while it did not affect the response of the TKCAT construct. Addition of rapamycin (Fig. 1C) led to the inhibition of hPRL3500CAT expression with an IC_{50} similar to that of FK506. Rapamycin caused, however, a similar inhibitory effect on TKCAT expression. Moreover, we found that rapamycin (1 μM) inhibits (to 51 ± 9%) expression of a β-galactosidase reporter gene linked to a cytomegalovirus promoter/enhancer (not shown), indicating a more general effect on transcription or translation in our system. GH_3 cell viability was not affected by addition of FK506, cyclosporin A or rapamycin in the concentration range used in Fig. 1.

SSDI 0014-5793(94)01416-7

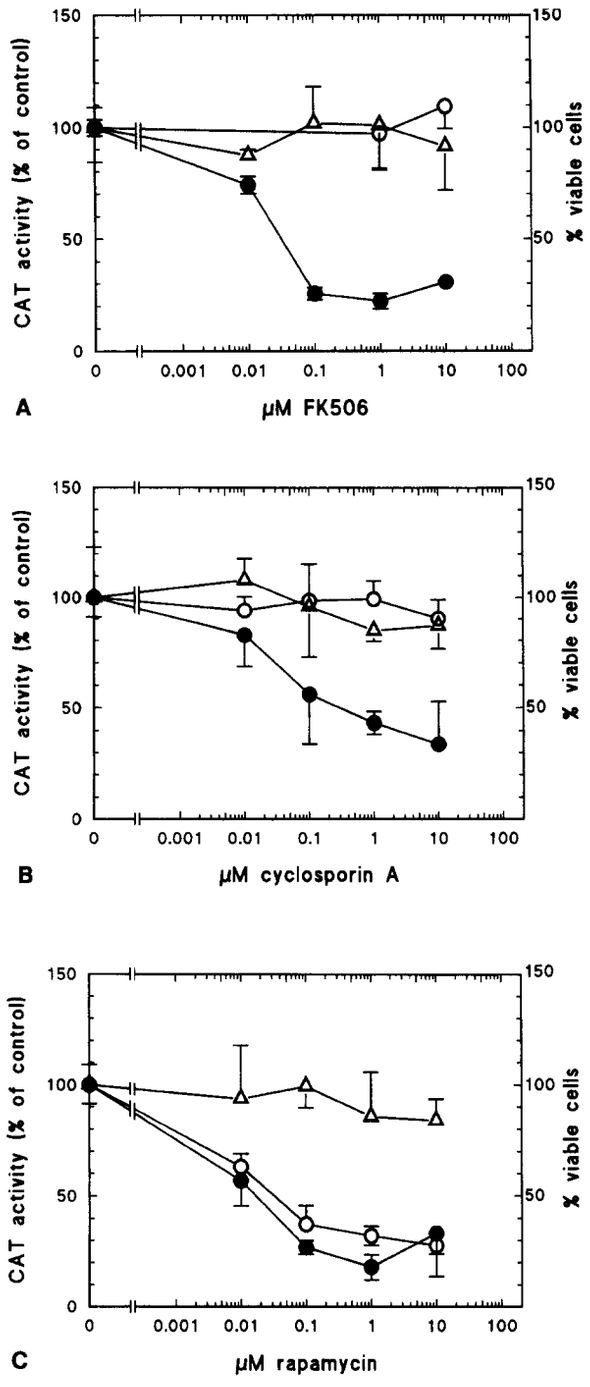

Fig. 1. Immunosuppressants inhibit hPRL-CAT transient expression. GH_3 pituitary tumor cells were transfected with the hPRL3500CAT construct (●) or with TKCAT (○). 2 h after transfection the indicated concentrations of FK506 (panel A), cyclosporin A (panel B) or rapamycin (panel C) were added. Toxicity of the compounds was tested in a Trypan blue exclusion assay (△).

3.2. *The proximal hPRL promoter is sufficient to mediate the effects of FK506*

To locate more precisely on the hPRL promoter the *cis*-elements which mediate its inhibition by immunosuppressants, we transfected GH_3 cells with a series of hPRLCAT deletion mutants (containing from 4700 bp to only 250 bp of the hPRL promoter) and monitored their transient expression in the presence or absence of 1 μM FK506. Fig. 2 shows that all these constructs responded similarly to the drug while the TKCAT control did not respond. This implies that the inhibitory effect of FK506 is mediated by the proximal 250 bp of the hPRL promoter which contains two types of regulatory elements: binding sites for the pituitary-specific transcription factor Pit-1, and the region extending from −115 to −85 bp named sequence A [9,12,13]. Since CAT expression driven by hPRL promoter constructs smaller than 250 bp is very low, it is difficult to further dissect the hPRL promoter when studying inhibitory effects.

3.3. *Immunosuppressants do not affect induction of hPRL gene expression by cAMP*

We showed previously that a small region of the proximal promoter (−164 to −34) of the hPRL gene can confer sensitivity to cAMP when fused to a cAMP-insensitive TKCAT construct [9]. Since the basal expression of this construct is driven by the TK promoter it is insensitive to FK506. Expression of this −164 to −34 hPRL-TKCAT construct was stimulated 15.9 fold by the addition of cAMP. This stimulation is not significantly altered (14.8 ± 1.3 fold stimulation) in the presence of 1 μM FK506 (not shown), indicating that FK506 does not interfere with the cAMP signaling pathway in pituitary cells.

4. Discussion

The present work shows that expression of the hPRL gene is potently inhibited by immunosuppressants in a pharmacological concentration range. In view of the immuno-activating role of prolactin [5], this inhibition might contribute to the mechanism of action of cyclosporin A, FK506 and rapamycin, and thus act complementary to the inhibition of T-lymphocyte activation. Interestingly, the glucocorticoid agonist and immunosuppressant dexamethasone was shown previously to inhibit hPRL gene expression 3 fold [14].

In analogy to the inhibition of interleukin-2 gene expression

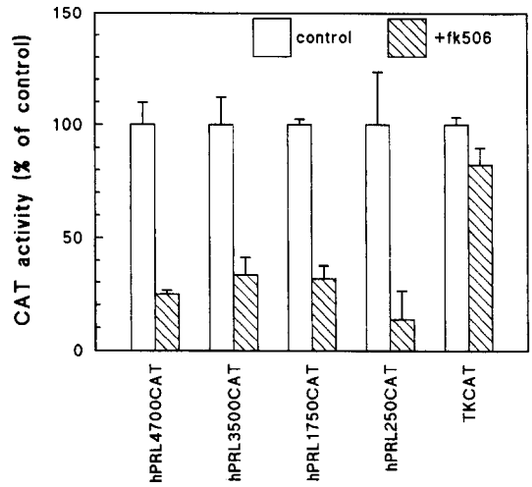

Fig. 2. The effect of FK506 is mediated by the proximal hPRL promoter. GH_3 pituitary tumor cells were transfected with hPRL promoter–CAT fusion genes containing 4700 bp, 3500 bp, 1750 bp, and 250 bp of the hPRL promoter as indicated, or with a TKCAT construct. 2 h after transfection, 1 μM of FK506 was added as indicated.

in T-lymphocytes, FK506- and cyclosporin A-induced inhibition of hPRL gene expression in the pituitary might be mediated by calcineurin inhibition. We observe the inhibition also with rapamycin, however, and this compound does not affect calcineurin activity [1–3]. The effect of FK506 and cyclosporin A is specific for hPRL gene expression whereas rapamycin also inhibits transcription or translation of the CAT and β-galactosidase reporter. This indicates that rapamycin inhibits hPRL gene expression via a different mechanism than FK506 or cyclosporin A.

The expression of the human prolactin gene is regulated by various hormones, factors and toxins, such as cAMP, Ca^{2+}, thyroliberin, epidermal growth factor and okadaic acid [9,12,13–15]. Most of the known intracellular second messenger pathways converge at the level of the proximal promoter, where their effects are mediated by two *cis*-acting elements: binding sites for the transcription factor Pit-1 and sequence A (−115 to −85). The binding activity of Pit-1 is controlled through phosphorylation/dephosphorylation [16]. The 100 kDa protein binding to sequence A [12] and its regulation remain to be further characterized. From the present work it appears that the inhibitory effect of immunosuppressants is also mediated via the proximal promoter containing both *cis*-acting elements.

Acknowledgements: The authors wish to thank Dr. V. Geenen for helpful discussions, Dr. B. Peers and Mrs. A.M. Nalda for making various plasmids available, Dr. K. Murato (Fujisawa GmbH) and Dr. L. Faucette (Smith-Kline Beecham) for the kind gift of FK506 and rapamycin, respectively. This work was supported by grants from the Belgian FRSM (3.4537.93), the Belgian Prime Minister's Office (Interuniversity Poles of Attraction PAI P3-044), the European Community (BIOT CT90-0188-C), and the Walloon Region (Convention 'Synergi', 1993).

References

[1] Liu, J., Farmer, J.D., Lane, W.S., Friedman, J., Weisman, I. and Schreiber, S.L. (1991) Cell 66, 807–815.
[2] Liu, J., Alberts, M.W., Wandless, T.J., Luan, S., Alberg, D.G., Belshaw, P.J., Cohen, P., Mackintosh, C., Klee, C.D. and Schreiber, S.L. (1992) Biochemistry 31, 3896–3901.
[3] Schreiber, S. and Crabtree G.R. (1992) Immunol. Today 13, 136–142.
[4] Brown, E.J., Albers, M.W., Shin, T.B., Ichikawa, K., Keith, C.T., Lane, W.S. and Schreiber, S.L. (1994) Nature 369, 756–758.
[5] Hooghe, R., Delhase, M., Vergani, P., Malur, A. and Hooghe-Peters, E.L. (1993) Immunol. Today 14, 212–214.
[6] Pullen, A.M., Wade, T., Marrack, P. and Kappler, J.W. (1990) Cell 61, 1365–1374.
[7] Russel, D.H., Mills, K.T., Talamantes, F.J. and Bern, H.A. (1988) Proc. Natl. Acad. Sci. USA 85, 7404–7407.
[8] Pellegrini, I., Lebrun, J.J., Ali, S. and Kelly, P.A. (1992) Mol. Endocrinol. 6, 1023–1031.
[9] Peers, B., Monget, P., Nalda, M.A., Voz, M.L., Berwaer, M., Belayew, A. and Martial, J.A.(1991) J. Biol. Chem. 266, 18127–18134.
[10] Gorman, C.M., Moffat, L.M. and Horward, B.H. (1982) Mol. Cell. Biol. 10, 4690–4700.
[11] Peers, B., Voz, M.L., Monget, P., Mathy-Hartert, M., Berwaer, M., Belayew, A. and Martial, J.A. (1990) Mol. Cell. Biol. 10, 4690–4700.
[12] Peers, B., Nalda, M.A., Monget, P., Voz, M., Belayew, A. and Martial, J.A. (1992) Eur. J. Biochem. 210, 53–58.
[13] Berwaer, M., Peers, B., Nalda, M.A., Monget, P., Davis, J.R.E., Belayew, A. and Martial, J.A. (1993) Mol. Cell. Endocrinol. 92, 1–7.
[14] Berwaer, M., Monget, P., Peers, B., Mathy-Hartert, M., Bellefroid, E., Davis, J.R.E., Belayew, A. and Martial, J.A. (1991) Mol. Cell. Endocrinol. 80, 53–64.
[15] Wera, S., Belayew, A. and Martial J.A. (1993) Mol. Endocrinol. 7, 965–971.
[16] Kapiloff, M.S., Farkash, Y., Wegner, M. and Rosenfeld, G.M. (1991) Science 253, 786–789