FEBS Letters 352 (1994) 1–3

FEBS 14471

Vitamin D influences gap junctional communication in C3H/10T 1/2 murine fibroblast cells

Wilhelm Stahl, Swantje Nicolai, Michael Hanusch, Helmut Sies*

Biologisch-Medizinisches Forschungszentrum and Institut für Physiologische Chemie I, Heinrich-Heine-Universität Düsseldorf, Postfach 101007, D-40001 Düsseldorf, Germany

Received 21 July 1994; revised version received 29 July 1994

Abstract Vitamin D3, cholecalciferol, induces cell–cell communication via gap junctions in murine fibroblasts (C3H/10T 1/2 cells) at concentrations between 0.01 and 1.0 μM, as assayed by the dye transfer method using Lucifer yellow CH. The extent of induction is similar to that obtained with the positive controls, canthaxanthin or retinoic acid, applied at 10 and 1 μM, respectively. Vitamin D2 also induces cell–cell communication. At elevated concentrations of vitamin D3 (5 μM) there is a suppression of gap junctional communication, reversible upon exposure to *all-trans* retinoic acid (1 μM) after removal of vitamin D3 from the medium. Conversely, communication between cells prestimulated with retinoic acid (1 μM) rapidly decreases when the retinoid is replaced by vitamin D3 (5 μM). The data demonstrate a role for vitamin D in the regulation of intercellular communication. This novel property of vitamin D may contribute to the antiproliferative effects of vitamin D exhibited in some types of cancer.

Key words: Vitamin D; Intercellular communication; Calciferol; C3H/10T 1/2 cell; Gap junction

1. Introduction

Vitamin D contributes to the regulation of gene expression through the metabolite, 1,25-$(OH)_2$ vitamin D3, a ligand of the vitamin D nuclear receptor (VDR). The vitamin D-responsive systems include genes governing oncogene and lymphokine expression and replication-linked genes critical for cell proliferation [1]. Growth regulatory functions in cells are also attributed to gap junctional communication which can be induced by retinoids [2–5] and carotenoids [6].

Recently, a signalling pathway has been described which is responsive to heterodimers of VDR and the retinoic X receptor α (RXRα). The DNA responsive element is synergistically activated by VDR and RXR, and the presence of both ligands, 1,25-$(OH)_2$ D_3 and 9-*cis* retinoic acid, is necessary for activation [7].

In the present study, we investigated the influence of vitamin D3 on cell–cell communication and its interplay with *all-trans* retinoic acid.

2. Materials and methods

2.1. Chemicals

Lucifer yellow CH and *all-trans* retinoic acid were purchased from Sigma (Deisenhofen, Germany), vitamins D3 and D2 from Fluka (Neu-Ulm, Germany). Canthaxanthin was a kind gift from Dr. J. Bausch, Hoffmann-La Roche (Basel, Switzerland). The cell proliferation kit I (MTT) was from Boehringer (Mannheim, Germany). All other chemicals were obtained from Merck (Darmstadt, Germany).

2.2. Cells and culture conditions

The murine embryo fibroblast C3H/10T 1/2 clone 8 cell line (ATCC, No. CCL 226) was cultured with fibroblast growth medium FGM from PromoCell (Heidelberg, Germany) supplemented with 10% fetal calf serum from Life Technologies (Eggenstein, Germany) in 35 × 10 mm dishes from Nunc (Wiesbaden, Germany). When confluence was reached, the fetal calf serum content was decreased to 3%, and the cells were then treated with the indicated concentrations of vitamins D3 or D2, dissolved in acetone. Canthaxanthin (10 μM) dissolved in tetrahydrofuran was used as a positive control [6]. The medium containing the respective compounds added for the experiment was exchanged every 3 days. Cells were washed twice with PBS before new medium was added. The final concentration of acetone or tetrahydrofuran in the culture medium was 0.5%. Cell viability was checked determining the activity of mitochondrial dehydrogenases (cell proliferation kit I) following the protocol provided by the supplier.

2.3. Gap junctional communication assay

On days 1, 3, 5, 8, 10 and 12 of the incubation, junctional communication was assayed by microinjection of the fluorescent dye Lucifer yellow CH (10% in 0.33 M LiCl, w/v) into cells of confluent cultures by means of a microinjector and micromanipulator (Eppendorf, Hamburg, Germany). The number of fluorescent neighbors of the injected cells was scored 5 min after injection and serves as an index of junctional communication [8]. The data plotted in the figures are mean values (± S.E.M.) of 10 individual injections of cells. Each experiment was repeated in triplicate with essentially the same results.

3. Results

Upon incubation of the cells with vitamin D3 the intercellular communication of C3H/10T 1/2 cells increased markedly above the solvent control (Fig. 1). Similar effects were obtained with vitamin D2 (data not shown). With concentrations of 0.01 and 0.1 μM the increase in the number of communicating cells was similar to that obtained with the positive control, canthaxanthin (10 μM). Gap junctional communication rose continuously up to day 12 of treatment.

Cell–cell communication decreased with higher concentrations of vitamin D3. At day 12, significantly less communication was observed in cultures treated with 1 μM vitamin D3 as compared to incubations containing lower (0.01 and 0.1 μM) concentrations. Moreover, almost complete loss of intercellular communication was achieved with 5 μM vitamin D3, reflected by a level significantly below the solvent control, already even on day 1 (Fig. 1).

No overt morphological changes or toxic effects, as checked by mitochondrial dehydrogenase activity, were detected as a result of vitamin D treatment at the concentrations employed.

After continuous inhibition (9 days) by exposure to vitamin D3 at 5 μM concentration, the intercellular communication of 10T 1/2 cells was restimulated when vitamin D3 in the medium

*Corresponding author. Fax: (49) (211) 311 3029.

SSDI 0014-5793(94)00858-2

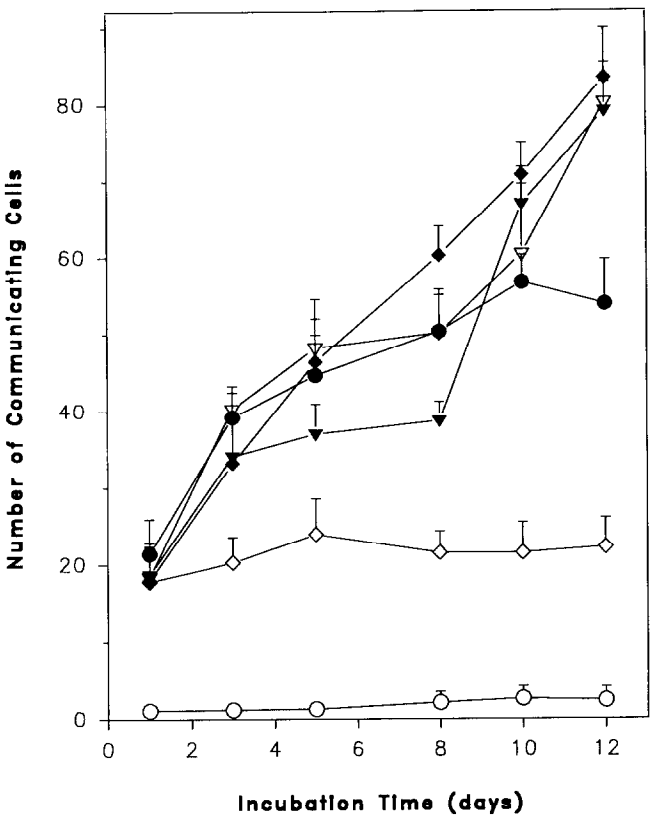

Fig. 1. Increase of gap junctional communication in murine C3H/10 T 1/2 fibroblasts upon treatment with different concentrations of vitamin D3. Vitamin D3 was added at the concentrations of 0.01 μM (▼), 0.1 μM (▽), 1.0 μM (●), and 5.0 μM (○). Solvent control was 0.5% acetone (◇). The positive control was 10 μM canthaxanthin in 0.5% tetrahydrofuran (♦). Solvent control for tetrahydrofuran is not shown, it was similar to 0.5% acetone.

was exchanged for 1 μM retinoic acid (Fig. 2, dotted arrow). Within 24 h a cell–cell communication in the range of the controls was observed, further increasing between days 10 and 14, the slope being comparable to that observed for 1 μM retinoic acid alone (top curve in Fig. 2). When the vitamin D3-containing medium was exchanged for solvent control on day 9, a slight increase in communication occurred as well, amounting to about 10 communicating cells on day 14 (not shown in Fig. 2).

Conversely, cell–cell communication in cells prestimulated with 1 μM retinoic acid (top curve in Fig. 2) was inhibited by exchanging retinoic acid in the medium for 5 μM of vitamin D3 on day 9 (Fig. 2, solid arrow). Solvent controls decreased slightly towards about 75 communicating cells on day 14 (not shown).

4. Discussion

Cell culture assays for chemicals with tumor-promoting or tumor-inhibiting activity based on the modulation of intercellular communication have been widely used [9]. The present work, to our knowledge, is the first report describing the influence of vitamin D on gap junctional communication, a process relevant in the regulation of cell growth and differentiation. The data suggest that calciferols and retinoids interplay in the regulatory process of gap junctional activity. It has been suggested that retinoic acid nuclear receptors are involved in the activation of the expression of connexins, a family of proteins forming the transmembrane channels [10]. The mechanism of action of the calciferols is yet unknown. As mentioned above, recently a signalling pathway has been described utilizing heterodimers between vitamin D receptors and retinoic acid receptors in the presence of their natural ligands [7]. Thus, it appears that vitamin D plays a role in the regulation of gap junctional communication through regulation of gene expression.

Furthermore, it should be noted that cellular calcium concentrations influence gap junctional permeability. With elevated concentrations of cytosolic calcium, less communication is observed [11]. The rapid suppression of cell–cell communication upon addition of high (5 μM) vitamin D3 concentrations (Fig. 1, day 1; Fig. 2, day 10 after removal of retinoic acid on day 9) could be due to increased calcium levels. This implies two different vitamin D-dependent regulatory mechanisms of intercellular communication, one being activatory, by regulation of connexin gene expression as described for retinoids. The other mechanism is inhibitory, observed at high vitamin D concentrations, possibly operating through changes in cellular calcium levels.

A variety of physiological functions have been attributed to vitamin D, predominantly related to calcium homeostasis, including intestinal calcium uptake, bone remodeling or mineral

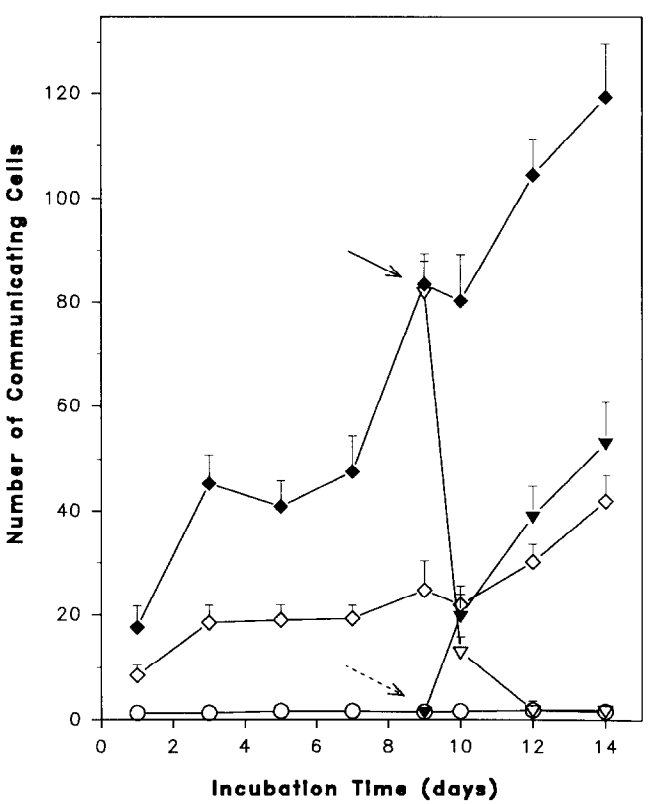

Fig. 2. Interplay of vitamin D3 and *all-trans* retinoic acid in modulating gap junctional communication. Cells were treated with 5 μM vitamin D3 (○), and on day 9 the medium was exchanged for 1 μM *all-trans* retinoic acid (▼), indicated by dotted arrow. Conversely, cells were treated with 1 μM *all-trans* retinoic acid (♦), and on day 9 the medium was exchanged for 5 μM vitamin D3 (▽), indicated by solid arrow. Solvent controls are indicated as (◇).

conservation in the kidney [12,13]. Vitamin D, its metabolites and several synthetic analogs have been shown to promote differentiation and inhibit the proliferation of several cancer cell lines in vitro, e.g. the human myeloid leukemia cell line HL-60 [14], breast cancer [15], colon cancer [16], and prostatic cancer cell lines [17,18]. In tumor-bearing animals, vitamin D derivatives suppressed tumor growth, inhibited metastasis, and prolonged survival times [19,20]. It appears feasible that some of these effects are mediated through modulation of intercellular communication.

Acknowledgements: Helpful discussions with Dr. W.A. Schulz are gratefully acknowledged. Supported by National Foundation for Cancer Research, Bethesda, MD, USA.

References

[1] Minghetti, P.P. and Norman, A.W. (1988) FASEB J. 2, 3043–3053.
[2] Loewenstein, W.R. and Kanno, Y. (1966) Nature 209, 1248–1249.
[3] Loewenstein, W.R. (1979) Biochim. Biophys. Acta 560, 1–65.
[4] Mehta, P.P., Bertram, J.S. and Loewenstein, W.R. (1986) Cell 44, 187–196.
[5] Yamasaki, H. (1990) Carcinogenesis 11, 1051–1058.
[6] Zhang, L.-X., Cooney, R.V. and Bertram, J.S. (1991) Carcinogenesis 12, 2109–2114.
[7] Carlberg, C., Bendik, I., Wyss, A., Meier, E., Sturzenbecker, L.J., Grippo, J.F. and Hunziker, W. (1993) Nature 361, 657–660.
[8] Stewart, W.W. (1978) Cell 14, 741–759.
[9] Budunova, I.V. and Williams, G.M. (1994) Cell Biol. Toxicol. 10, 71–116.
[10] Hossain, M.Z., Zhang, L.-X. and Bertram, J.S. (1993) Progr. Cell Res. 3, 301–309.
[11] De Mello, W.C. (1975) J. Physiol. 250, 231–245.
[12] Reichel, H., Koeffler, H.P. and Norman, A.W. (1989) N. Engl. J. Med. 320, 980–991.
[13] DeLuca, H.F. (1992) Ann. NY Acad. Sci. 669, 59–69.
[14] Tanaka, H., Abe, E., Miyaura, C., Kuribayashi, T., Konno, K., Nishii, Y. and Suda, T. (1982) Biochem. J. 204, 713–719.
[15] Colston, K.W., Mackay, A.G., James, S.Y., Binderup, L., Chander, S. and Coombes, R.C. (1992) Biochem. Pharmacol. 44, 2273–2280.
[16] Thomas, M.G., Tebbutt, S. and Williamson, R.C.N. (1992) Gut 33, 1660–1663.
[17] Skowronski, R.J., Peehl, D.M. and Feldman, D. (1993) Endocrinology 132, 1952–1960.
[18] Peehl, D.M., Skowronski, R.J., Leung, G.K., Wong, S.T., Stamey, T.A. and Feldman, D. (1994) Cancer Res. 54, 805–810.
[19] Sato, T., Takusagawa, K., Asoo, N. and Konno, K. (1984) Br. J. Cancer 50, 123–125.
[20] Eisman, J.A., Barkla, D.H. and Tutton, P.J.M. (1987) Canc. Res. 47, 21–25.