Reprod Nutr Dev (1997) 37, 3-12

Original article

Decrease in CGRP and CT levels either contained in or released by CA-77 C cells after combined treatments with 1,25-dihydroxyvitamin D_3 analogues and 9-*cis* retinoic acid

Y Lamari, JM Garel *

Endocrinologie moléculaire et métabolique (EA n° 1526 DRED), Université Pierre-et-Marie-Curie, 4, place Jussieu, 75252 Paris cedex 05, France

(Received 22 March 1996; accepted 31 July 1996)

Summary — This study examined the action of 9-*cis* retinoic acid and 1,25-dihydroxyvitamin D_3 analogues (KH 1060, EB 1089 and MC 903) on the release of calcitonin (CT) and calcitonin gene-related peptide (CGRP) in the rat C cell line CA-77. This cell line mainly secretes CGRP. Using radioimmunoassays (RIAs) for CT and CGRP, we measured the release of both peptides in the culture medium as well as the amount of these proteins contained in the CA-77 C cells. 9-*cis* retinoic acid decreased the release of both CGRP and CT dose-dependently in the range between 1 nM and 1 μM. The half-effective dose was 10 nM. The treatment of CA-77 C cells with 0.1 μM calcitriol alone only slightly decreased the release of both CT and CGRP. The increase in the amount of CT and CGRP released by the action of 1 μM dexamethasone was reduced by 1 μM 9-*cis* retinoic acid, and this effect was enhanced by the addition of 0.1 μM calcitriol or KH 1060, EB 1089 and MC 903. When the C cells were continuously stimulated by dexamethasone, after 6 days of exposure to the combined treatment with calcitriol analogues + 9-*cis* retinoic acid, there was a greater decrease in the amount of CGRP contained in the C cells than after treatment with 9-*cis* retinoic alone. Our data suggested that combined treatment with retinoic acid and calcitriol analogues exerted a stronger inhibition on the amounts of the two peptides either contained in the cells or released in the medium than each hormone alone.

CA-77 C cell / cellular CT content / CT release / cellular CGRP content / CGRP release / 9-*cis* retinoic acid / calcitriol / calcitriol analogues

* Correspondence and reprints
Tel: (33) 01 44 27 32 47; fax: (33) 01 44 07 15 85; e-mail: jean-michel-garel@snv.jussieu.fr

Résumé — Diminution du contenu cellulaire et de la libération de CT et de CGRP par les cellules « C » CA-77 sous l'effet d'un traitement combinant l'acide rétinoïque 9-*cis* et des analogues du 1,25-dihydroxycholécalciférol. Cette étude porte sur l'action de l'acide rétinoïque 9-*cis* et des analogues du calcitriol (KH 1060, EB 1089, et MC 903) sur la libération dans le milieu de culture de la calcitonine (CT) et du peptide alternatif du gène de la calcitonine (CGRP) dans une lignée tumorale de cellules « C » d'origine murine, les cellules CA-77. Cette lignée exprime majoritairement le CGRP. Le contenu cellulaire et la sécrétion dans le milieu de culture de CT et de CGRP ont été dosés par radioimmunologie (RIA). Pour les concentrations comprises entre 1 nM et 1 µM, l'acide rétinoïque 9-*cis* entraîne une diminution dose-dépendante de la libération de la CT et du CGRP. L'inhibition de moitié de la valeur maximale est observée sous l'effet d'un traitement à 10 nM. Le traitement des cellules CA-77 avec du calcitriol 0,1 µM seul n'entraîne qu'une légère diminution de la libération de CT et de CGRP. L'augmentation de la quantité des deux peptides libérée sous l'effet de la dexaméthasone 1 µM est amoindrie par l'acide rétinoïque 9-*cis* 1 µM et cet effet inhibiteur est amplifié par l'addition de calcitriol ou de KH 1060 ou du EB 1089 ou encore du MC 903 aux doses de 0,1 µM. Le traitement combiné des cellules C, pendant 6 jours, par de la dexaméthasone, de l'acide rétinoïque 9-*cis* et des analogues du calcitriol diminue plus fortement le contenu cellulaire en CGRP qu'un traitement à l'acide rétinoïque 9-*cis* seul. Nos résultats suggèrent qu'un traitement simultané des cellules C avec de l'acide rétinoïque 9-*cis* et des analogues du calcitriol provoque une plus forte inhibition du contenu cellulaire et de la libération des peptides issus du gène de la CT que chaque hormone utilisée seule.

cellules « C » CA-77 / CT / CGRP / contenu cellulaire / sécrétion / acide rétinoïque 9-*cis* / calcitriol / analogues du calcitriol

INTRODUCTION

The CA-77 C cell line is derived from a rat medullary thyroid carcinoma (Roos et al, 1979), and mainly expresses calcitonin gene-related peptide (CGRP) as a product of the calcitonin (CT) gene. We have already shown that the combined treatment of CA-77 C cells with µM doses of all-*trans* retinoic acid and 0.1 µM calcitriol resulted in a greater inhibition of CGRP mRNA production than each compound alone, and consequently led to a decrease in the amount of CGRP contained in the cells and in the amount released (Lamari et al, 1994). Since 9-*cis* retinoic acid is the specific ligand of retinoid X receptors (Heyman et al, 1992), we investigated here the effects of a combination of calcitriol and 9-*cis*-retinoic acid on the amounts of CT and CGRP contained in the cells as well as that released by the CA-77 C cells. The use of 1,25-dihydroxyvitamin D_3 (calcitriol) analogues (EB 1089 and MC 903), which are less hypercalcemic than calcitriol plus retinoic acid at inhibiting CT gene expression, will be of potential value for the treatment of human medullary thyroid carcinoma. To this end, we compared the effects of such calcitriol analogues (EB 1089 and KH 1060), which are considerably more active in vitro in the inhibition of cell growth (Mathiasen et al, 1993) to the action of calcitriol.

MATERIALS AND METHODS

Cell culture

The CA-77 cells were maintained in DMEM/Ham's F10 (1:1) (Gibco/BRL, France) supplemented with 10 µg/mL insulin (Sigma), 3.10^{-8} M sodium selenite (Gibco/BRL, France),

5 µg/mL transferrin (Gibco/BRL, France), 110 mg/L sodium pyruvate (Gibco/BRL, France) and a mixture of antibiotics (100 units/mL penicillin + 100 µg/mL streptomycin; Gibco/BRL, France). The cells were plated at an initial density of 4.10^4 cells/cm^2 in a medium containing DMEM/Ham's F10 (1:1), 10% heat-inactivated foetal calf serum (J Boy, Reims, France), 110 mg/L sodium pyruvate, 100 units/mL penicillin and 100 µg/mL streptomycin. After 48 h of plating, the medium was changed and replaced by the growth medium described earlier. The growth medium was changed at 48 h intervals. Dexamethasone (Sigma, Saint Louis, MO, USA) and 9-*cis* retinoic acid (Hoffmann-La Roche, Basel, Switzerland) were dissolved in absolute ethanol and then in PBS to reach a final ethanol concentration of 0.005% in the growth medium. 1,25-$(OH)_2D_3$, KH 1060, EB 1089 and MC 903 (Leo Pharmaceutical Products, Ballerup, Denmark) were dissolved in absolute isopropanol and then diluted with ethanol and growth medium to give no more than 0.01% ethanol in the culture medium. Controls with the same volume of vehicle were used in each case.

Radioimmunoassays (RIAs)

The cell culture medium was collected and treated as previously described (Wind et al, 1993) to measure the CT and CGRP levels. The amount of peptide (CT and CGRP) contained in the C cells was also measured by RIAs. A volume of 400 µL 0.1 N HCl was added to the culture dishes. The cells were then scraped and sonicated for 15 s and left to stand 16 h at 4 °C. The CT RIA has already been reported (Heath and Sizemore, 1982; Jousset et al, 1988). The G813 antibody (goat antiserum raised against synthetic human CT) was a gift of Dr H Heath (Mayo Clinic and Mayo Foundation, Rochester, MN, USA), and the detection limit of the assay was 3.9 pg per tube. This assay used ^{125}I-labelled human CT and unlabelled synthetic human CT (Ciba-Geigy, Basel, Switzerland) as standards. The results are expressed as ng equivalent human CT per mg protein since the protein content of the culture dishes was determined by the Lowry method (Lowry et al, 1951). Intra-assay variations for the CT RIA were 5% and inter-assay variations were 10%. For the CGRP, the RIA used has been described previously (Maubras et al, 1993; Lamari et al, 1995). We used synthetic human CGRP (Sigma) as the standard, a sheep antibody raised against synthetic human CGRP (kindly donated by Dr MS Moukthar, Unité INSERM 349, Paris, France) diluted 1:100 000, and ^{125}I-iodohistidyl human CGRP from Amersham (Les Ulis, France). The tubes were preincubated for 4 days at 4 °C and then incubated for 3 days at 4 °C in the presence of labelled CGRP. The bound to free fractions were separated by adding 0.15 mL of dextran charcoal suspension buffer to each assay tube (0.5 mL). After centrifugation at 2 000 g for 20 min, the supernatant was discarded. The detection limit of this assay was 10 pg per tube with intra-assay variations of 6%, and inter-assay variations of 11%.

Statistical analysis

All probabilities were calculated using the Mann-Whitney U test for ranked non-parametric data. All data are presented as the means ± standard error of the mean four determinations.

RESULTS

A log dose-dependent inhibition of the amount of both CT and CGRP released in the culture medium was observed when 9-*cis* retinoic acid was added for 3 days (fig 1). The CGRP secretion was decreased for a dose of 1 nM, and the CT secretion decreased for 10 nM. The half-effective dose of 9-*cis* retinoic acid was 10 nM (fig 1). After 3, 5 and 7 days of exposure with 1 µM 9-*cis* retinoic acid, the secretion of both CT and CGRP continued to be reduced by around 50% (figs 2 and 3). The addition of 0.1 µM calcitriol alone to the culture medium induced a slight decrease in the amount of CT released (–15%) by the CA-77 C cells (fig 3). The decrease in CGRP secre-

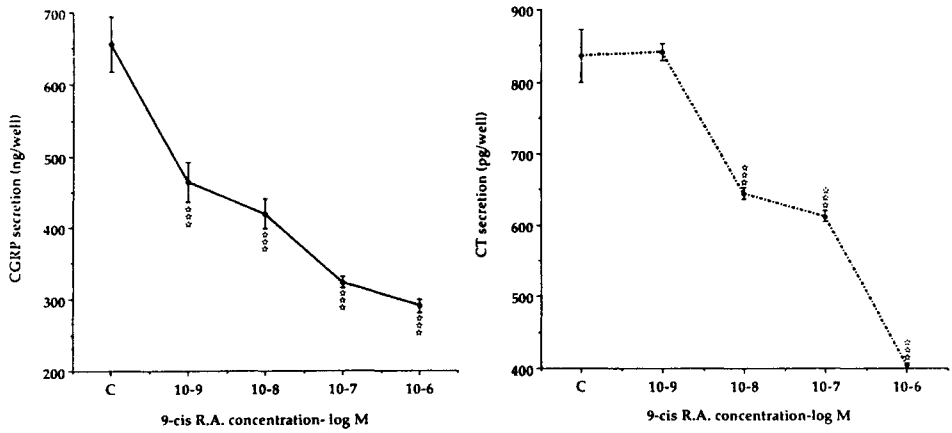

Fig 1. Log-dose effect of 9-*cis* retinoic acid (RA) on calcitonin (CT) and calcitonin gene-related peptide (CGRP) release in the culture medium after 3 days of treatment. Medium containing fresh hormones was changed every 48 h and cells were continuously exposed to the hormone. ☆☆☆ $P < 0.001$ are significantly different from controls (C).

tion was only significant on days 3 and 7 (fig 2). The amount of CGRP and CT contained in the C cells remained unchanged on day 7 after exposure to 9-*cis* retinoic acid and calcitriol. Co-treatment of CA-77 C cells by dexamethasone and 9-*cis* retinoic acid reduced the usual increase in the release of both CT and CGRP induced by 1 μM dexamethasone

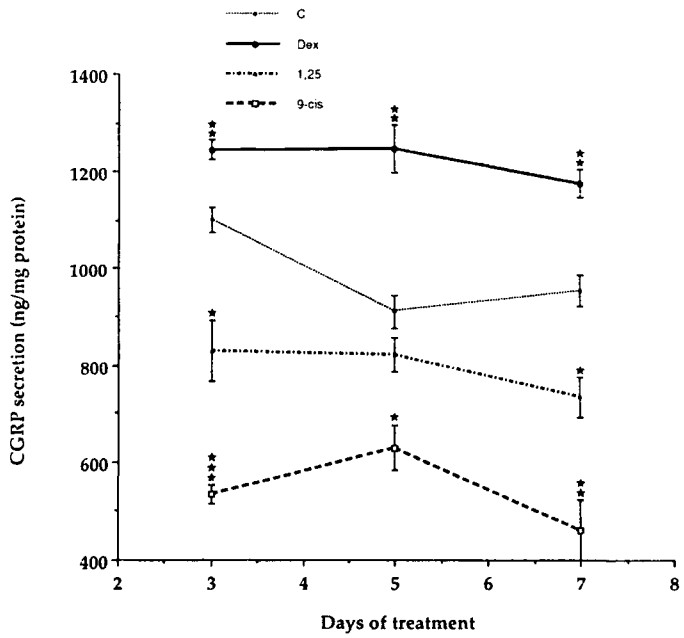

Fig 2. Effect of 9-*cis* retinoic acid (9-*cis*), dexamethasone (Dex) and calcitriol (1,25) on calcitonin gene-related peptide (CGRP) release by CA-77 cells. Medium containing fresh hormones was changed every 48 h, and cells were continuously exposed to the hormones. C: controls; 9-*cis*: 1 μM; Dex: 1 μM; 1,25: 0.1 μM. Means ± SEM are given for four culture dishes. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ are significantly different from controls.

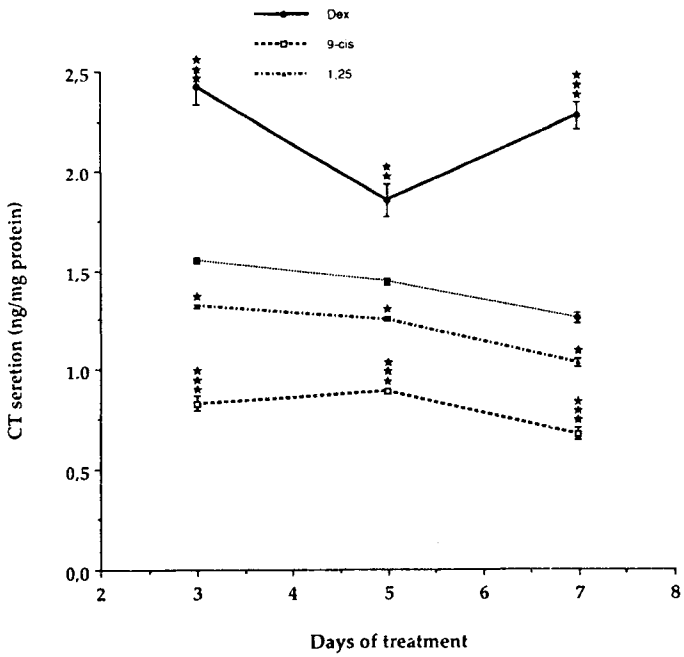

Fig 3. Effect of 9-*cis* retinoic acid (9-*cis*), dexamethasone (Dex) and calcitriol (1,25) on calcitonin (CT) release by CA-77 cells. Medium containing fresh hormones was changed every 48 h and cells were continuously exposed to the hormones. C: controls; 9-*cis*: 1 μM; Dex: 1 μM; 1,25: 0.1 μM. Means ± SEM are given for four culture dishes. ★ $P < 0.05$, ★★ $P < 0.01$, ★★★ $P < 0.001$ are significantly different from controls.

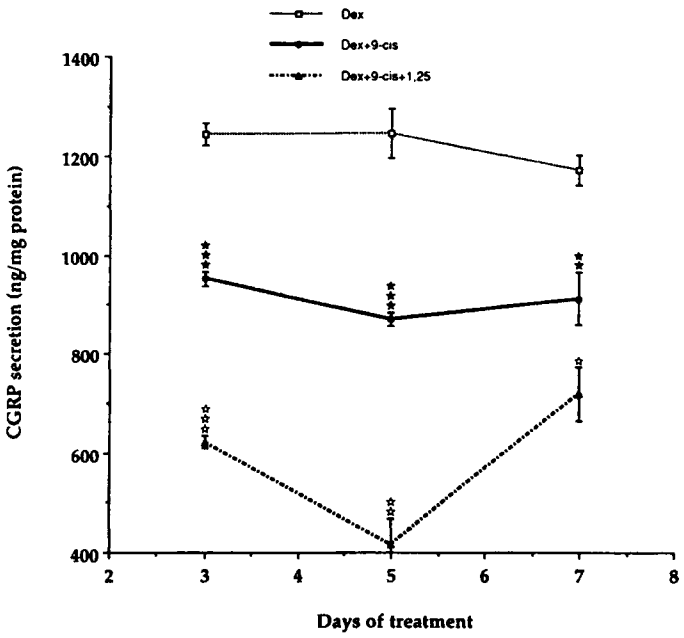

Fig 4. Effect of dexamethasone (Dex), dexamethasone + 9-*cis* retinoic acid (Dex + 9-*cis*) and dexamethasone + 9-*cis* + calcitriol (Dex + 9-*cis* + 1,25) on calcitonin gene-related peptide (CGRP) release by CA-77 C cells. Medium containing fresh hormones was changed every 48 h and cells were continuously exposed to the hormones. 9-*cis*: 1 μM; Dex: 1 μM; 1,25: 0.1 μM. Means ± SEM are given for four culture dishes. ★★ $P < 0.01$, ★★★ $P < 0.001$ from the Dex group; ☆ $P < 0.5$, ☆☆ $P < 0.01$, ☆☆☆ $P < 0.01$ from the Dex + 9-*cis* group.

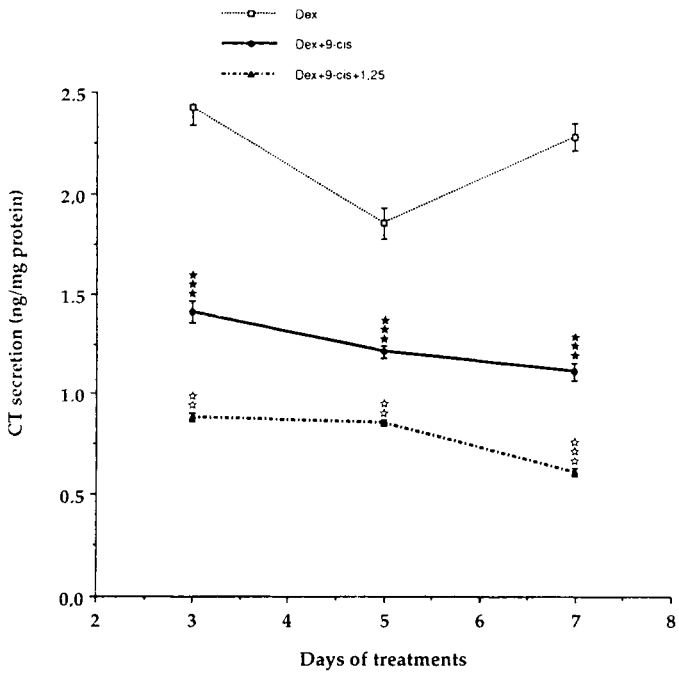

Fig 5. Effect of dexamethasone (Dex), dexamethasone + 9-*cis* retinoic acid (Dex + 9-*cis*) and dexamethasone + 9-*cis* + calcitriol (Dex + 9-*cis* + 1,25) on calcitonin (CT) release by CA-77 C cells. Medium containing fresh hormones was changed every 48 h, and cells were continuously exposed to the hormones. 9-*cis*: 1 μM; Dex: 1 μM; 1,25: 0.1 μM. Means ± SEM are given for four culture dishes. ★★★ $P < 0.001$ are significantly different from the Dex group; ☆☆ $P < 0.01$, ☆☆☆ $P < 0.001$ from the Dex + 9-*cis* group.

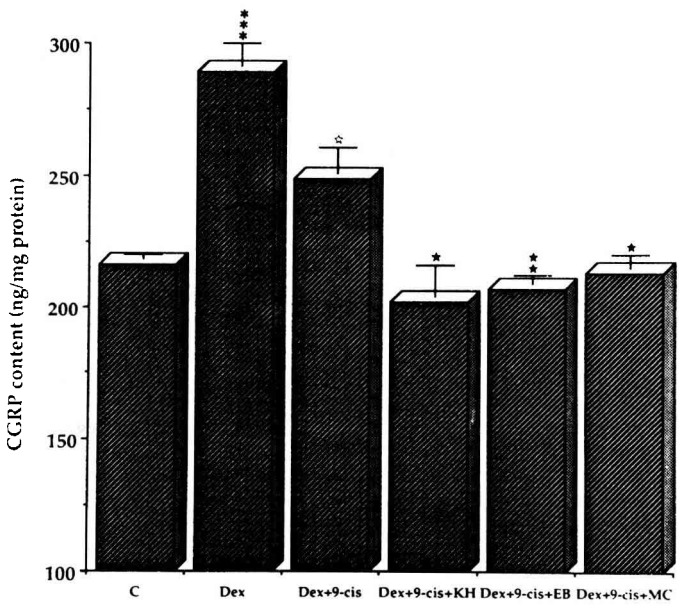

Fig 6. Effect of dexamethasone (Dex), dexamethasone + 9-*cis* retinoic acid (Dex + 9-*cis*), dexamethasone + 9-*cis* + KH 1060 (Dex + 9-*cis* + KH), dexamethasone + 9-*cis* + EB 1089 (Dex + 9-*cis* + EB) and dexamethasone + 9-*cis* + MC 903 (Dex + 9-*cis* + MC) on the calcitonin gene-related peptide (CGRP) content of CA-77 C cells. Medium containing fresh hormones was changed every 48 h. 9-*cis*: 1 μM; Dex: 1 μM; KH: 0.1 μM; EB: 0.1 μM; MC: 0.1 μM. Means ± SEM are given for four culture dishes. *** $P < 0.001$ from controls; ☆ $P < 0.05$ is significantly different for the Dex group; ★ $P < 0.05$, ★★ $P < 0.01$ from the Dex + 9-*cis* group.

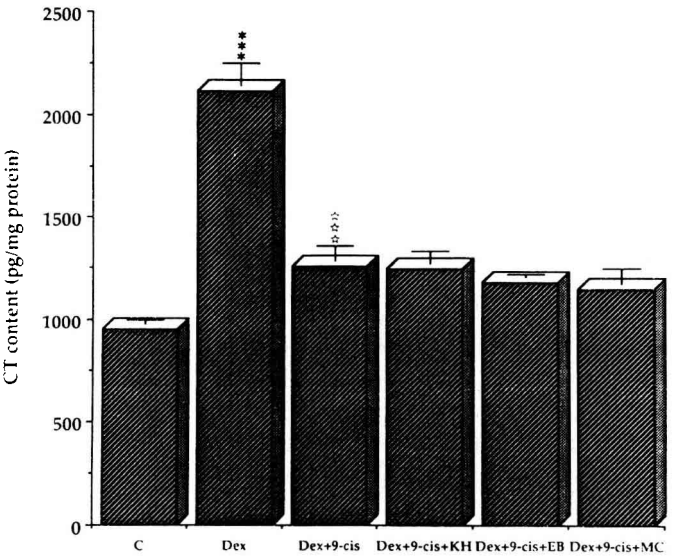

Fig 7. Effect of dexamethasone (Dex), dexamethasone + 9-*cis* retinoic acid (Dex + 9-*cis*), dexamethasone + 9-*cis* + KH 1060 (Dex + 9-*cis* + KH), dexamethasone + 9-*cis* + EB 1089 (Dex + 9-*cis* + EB) and dexamethasone + 9-*cis* + MC 903 (Dex + 9-*cis* + MC) on the calcitonin (CT) content of CA-77 C cells. Medium containing fresh hormones was changed every 48 h. 9-*cis*: 1 μM; Dex: 1 μM; KH: 0.1 μM; EB: 0.1 μM; MC: 0.1 μM. Means ± SEM are given for culture dishes. *** $P < 0.001$ is significantly different from controls; ☆☆☆ $P < 0.001$ from the Dex group.

(figs 4 and 5). The amount of CGRP and CT contained in the C cells increase with the dexamethasone treatment but was reduced by the addition of 9-*cis* retinoic acid (figs 6 and 7). This effect was already observed after 4 days of exposure to the hormones (data not shown), and again after 7 days of treatment (894 ± 48 in controls vs 1 240 ± 19 in the dexamethasone group [$P < 0.001$] vs 998 ± 7 pg CT/mg protein in the dexamethasone + 9-*cis* retinoic group [$P < 0.001$] and 274 ± 17 in controls vs 430 ± 19 [$P < 0.001$] in the dexamethasone group vs 357 ± 14 ng CGRP/mg protein in the dexamethasone + 9-*cis* retinoic acid group [$P < 0.01$]. A further decrease in the amount of CT and CGRP released in the culture medium was observed with the combined treatment of calcitriol and 9-*cis* retinoic acid (figs 4 and 5). Moreover, the amount of both CT and CGRP contained in the cells was slightly more decreased after 7 days of exposure to the hormones (865 ± 50 vs 998 ± 7 pg

Table I. Effects of dexamethasone, 9-*cis* retinoic acid and dihydroxy vitamin D_3 analogues on the protein content (in mg) of CA-77 C cells.

	Controls	*Dex*	*Dex* + 9-cis	*Dex* + 9-cis + *KH 1060*	*Dex* + 9-cis + *EB 1089*	*Dex* + 9-cis + *MC 903*
4 days	0.84 ± 0.01	0.78 ± 0.02*	0.80 ± 0.02	0.82 ± 0.0	0.85 ± 0.02	0.87 ± 0.03
6 days	1.00 ± 0.01	0.89 ± 0.02**	0.85 ± 0.03	0.84 ± 0.04	0.85 ± 0.01	0.84 ± 0.03

The cells were continuously exposed to hormones for 4 or 6 days. The medium with fresh hormones was changed every 48 h. Dexamethasone (Dex): 1 μM; 9-*cis* retinoic acid (9-*cis*): 1 μM; KH 1060: 0.1 μM; EB 1089: 0.1 μM; MC 903: 0.1 μM. Means ± SEM are given for four culture dishes. * $P < 0.05$; ** $P < 0.001$ are significantly different from controls.

CT/mg protein in the dexamethasone + 9-*cis* retinoic acid group [$P < 0.05$]; 280 ± 12 vs 357 ± 14 ng CGRP/mg protein in the dexamethasone + 9-*cis* retinoic acid group [$P < 0.05$].

At the doses used, the hormones had no effect on cell viability or cellular detachment. Dexamethasone at 1 μM induced a 10% decrease in the protein content of the cells after 4 and 6 days of continuous exposure (table I). Neither 9-*cis* retinoic acid nor the calcitriol analogues (KH 1060, EB 1089 and MC 903) changed the protein content of the cells (table I).

The combined treatments with 0.1 μM KH 1060, EB 1089 and MC 903 + 1 μM 9-*cis* retinoic acid gave similar results to those observed with calcitriol. After 6 days of exposure, the inhibition by the two hormones of dexamethasone-induced CGRP and CT release was greater than that observed after treatment with 9-*cis* retinoic acid alone (figs 8 and 9). This effect was already observed after 4 days of treatment for CGRP release (fig 8). Similar effects were observed for each calcitriol analogue. When the CA-77 cells were treated for 6 days with 9-*cis* retinoic acid in the presence of dexamethasone, the increase in the amount of CGRP contained in the cells was partially inhibited (fig 6). The combined treatment with KH 1060, EB 1089 or MC 903 and + 9-*cis* retinoic acid completely inhibited the dexamethasone induction (fig 6) since the amount of CGRP contained in the C cells did not differ from the controls. Such observations were not true, however, for the amount of CT contained in the C cells (fig 7). This amount did not decrease further with the addition of the calcitriol analogue.

DISCUSSION

We have previously shown that all-*trans* retinoic acid (50 μM) markedly decreased both the biosynthesis and secretion of CT and

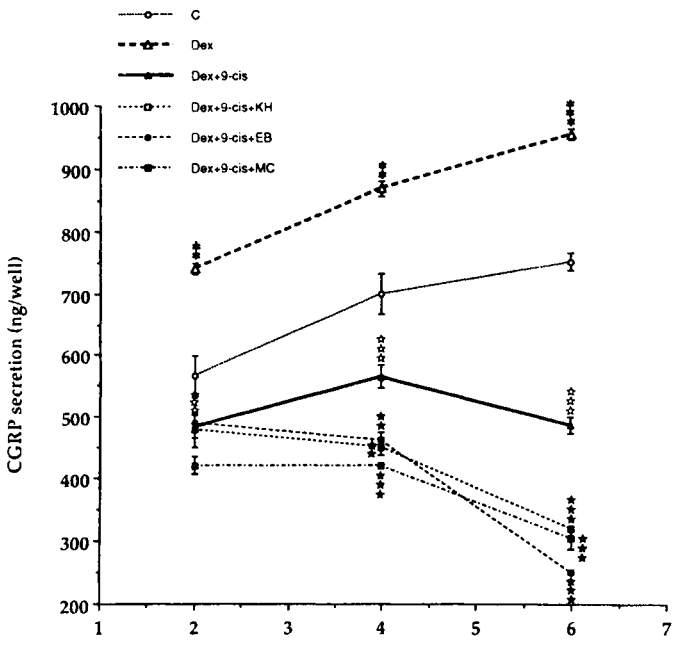

Fig 8. Effect of dexamethasone (Dex), dexamethasone + 9-*cis* retinoic acid (Dex + 9-*cis*), dexamethasone + 9-*cis* + KH 1060 (Dex + 9-*cis* + KH), dexamethasone + 9-*cis* + EB 1089 (Dex + 9-*cis* + EB) and dexamethasone + 9-*cis* + MC 903 (Dex + 9-*cis* + MC) on the calcitonin gene-related peptide (CGRP) release by CA-77 C cells. Medium containing fresh hormones was changed every 48 h. 9-*cis*: 1 μM; Dex: 1 μM; KH: 0.1 μM; EB: 0.1 μM; MC: 0.1 μM. Means ± SEM are given for four culture dishes. ** $P < 0.01$, *** $P < 0.001$ are significantly different from controls; ☆☆ $P < 0.01$, ☆☆☆ $P < 0.001$ from the Dex group; ★ $P < 0.05$, ★★ $P < 0.01$, ★★★ $P < 0.001$ from the Dex + 9-*cis* group.

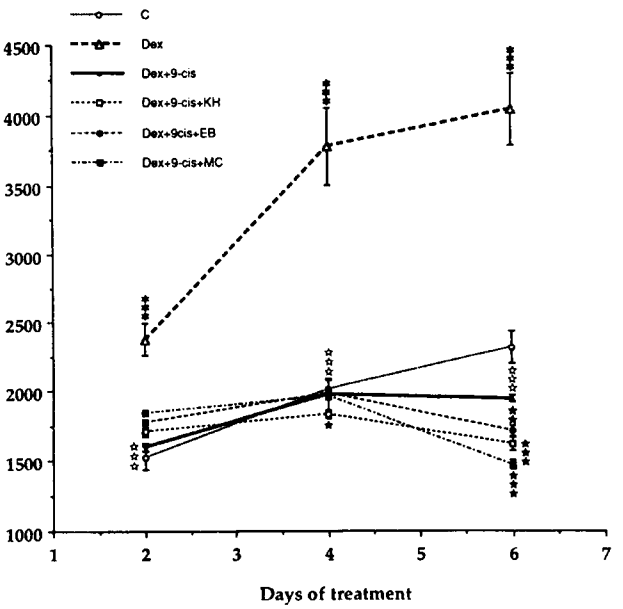

Fig 9. Effect of dexamethasone (Dex), dexamethasone + 9-*cis* retinoic acid (Dex + 9-*cis*), dexamethasone + 9-*cis* + KH 1060 (Dex + 9-*cis* + KH), dexamethasone + 9-*cis* + EB 1089 (Dex + 9-*cis* + EB) and dexamethasone + 9-*cis* + MC 903 (Dex + 9-*cis* + MC) on the calcitonin (CT) release by CA-77 C cells. Medium containing fresh hormones was changed every 48 h. 9-*cis*: 1 μM; Dex: 1 μM; KH: 0.1 μM; EB: 0.1 μM; MC: 0.1 μM. Means ± SEM are given for four culture dishes. *** $P < 0.001$ are significantly different from controls; ☆☆☆ $P < 0.001$ from the Dex group; * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ from the Dex + 9-*cis* group.

CGRP in CA-77 C cells as a consequence of a decrease in the amount of mRNA present (Lamari et al, 1994). A slight decrease in the release of both hormones in the culture medium was observed with a lower dose (5 μM) after 6 days of exposure to all-*trans* retinoic acid (Lamari et al, 1995). The present results demonstrated that CA-77 cells are also sensitive to 9-*cis* retinoic acid since the release of both CT and CGRP were decreased with a half-effective dose of 10 nM. With 1 μM 9-*cis* retinoic acid, a clear-cut decrease in the release of CT and CGRP in the culture medium occurred after 2, 3, 5, 6 and 7 days of continuous exposure to the hormone. No changes were observed in the amount of both peptides contained in the cells. Recent evidence suggests that isomerization of all-*trans* retinoic acid to 9-*cis* retinoic acid occurs in the cells (Urbach and Rando, 1994). 9-*cis* retinoic acid, a stereoisomer of all-*trans*, is a specific ligand for retinoic X receptors (RXR) (Heyman et al, 1992). It has also been found to bind retinoic acid receptors (RAR) (Schräder et al, 1993), which are well known to be activated by all-*trans* retinoic acid. Since 9-*cis* retinoic acid mimicked the effects of all-*trans* retinoic acid in our experiments, we could not rule out the involvement of two types of retinoic acid receptors (RXR and RAR) in our experiments.

An interesting observation was that 9-*cis* retinoic acid reduced the dexamethasone-induced release of CT and CGRP. A similar effect was observed on the amount of both peptides contained in the C cells. Such data were in agreement with those obtained with all-*trans* retinoic acid (Collignon et al, 1992; Lamari et al, 1994). One of the most stricking results was enhancement of the inhibitory effect due to the combined treatment with the calcitriol analogues and 9-*cis* retinoic acid on the amounts of CGRP and CT, both contained in the C cells and that which was released. These data were in agreement with our previous studies performed with calcitriol and all-*trans* retinoic acid (Lamari et al, 1994), and they were related to a change in CT gene expression (Lamari et al, 1994). Our present results may provide an example of a stronger

inhibition of the CT gene by what is likely a coupled action of calcitriol analogues and 9-*cis* retinoic acid to form an heterodimer receptor rather than an homodimer receptor.

ACKNOWLEDGMENTS

We are grateful to Pr BA Ross (University of Miami, FL, USA) for the gift of the CA-77 C cells. We thank Dr L Binderup (Leo Pharmaceutical Products, Ballerup, Denmark) for the gift of MC 903, EB 1089, KH 1060 and calcitriol. We thank Pr HH Heath (Mayo Clinic and Mayo Foundation, Rochester, MN, USA) for the gift of the G813 antibody. The CGRP antibody was kindly donated by Dr MS Moukhtar (Unité INSERM 349, Paris, France). The synthetic 9-*cis* retinoic acid was kindly donated by Hoffmann-La Roche (Basel, Switzerland). The assistance of R Bensalem and P Ranguis is gratefully acknowledged. This work was supported by a grant from the Fondation pour la Recherche Médicale.

REFERENCES

Collignon H, Laborie C, Tahri EH, El M'Selmi A, Garel JM (1992) Effects of dexamethasone, calcium and 1,25-dihydroxycholecalciferol on calcitonin and calcitonin gene-related peptide mRNA levels from the CA-77 cell line. *Thyroid* 2, 361-365

Heath H, Sizemore GW (1982) Radioimmunoassay for calcitonin. *Clin Chem* 28, 1219-1226

Heyman RA, Mangelsdorf DJ, Dyck JA, Stein RB, Eichele G, Evans RM, Thaller C (1992) 9-*cis* retinoic acid is a high affinity ligand for the retinoid X receptor. *Cell* 68, 397-406

Jousset V, Besnard P, Segond N, Jullienne A, Garel JM (1988) Potassium administration and calcitonin mRNA level in the rat. *Mol Cell Endocrinol* 59, 165-169

Lamari Y, Tahri EH, Collignon H, Garel JM (1994) Steroid hormones and retinoic acid interact in the regulation of calcitonin and calcitonin gene-related peptide secretion and messenger ribonucleic acid levels in CA-77 cells. *Cell Mol Biol* 40, 541-550

Lamari Y, Ghorbel M, Garel JM (1995) Effects of 17 β-estradiol on calcitonin and calcitonin gene-related peptide secretions and contents in a murine medullary thyroid carcinoma C-cell line (CA-77). *Reprod Nutr Dev* 35, 655-661

Lowry OH, Roebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the folin phenol reagent. *J Biol Chem* 193, 265-275

Mathiasen IS, Colston KW, Binderup L (1993) EB 1089, a novel vitamin D analogue, has strong antiproliferative and differentiation inducing effects on cancer cells. *J Steroid Biochem Mol Biol* 46, 365-371

Maubras L, Taboulet J, Pidoux E, Lasmoles F, Jullienne A, Milhaud G, Benson AA, Moukhtar MS, Cressent M (1993) Expression of CGRP mRNAs in the pink salmon, *Oncorhynchus gorbuscha. Peptides* 14, 977-981

Roos BA, Yoon MJ, Frelinger AL, Pensky RS, Birnbaum RS, Lambert PW (1979) Tumor growth and calcitonin during serial transplantation of rat medullary thyroid carcinoma. *Endocrinology* 105, 27-32

Schräder M, Bendik I, Becker-André M, Carlberg C (1993) Interaction between retinoic acid and vitamin D signaling pathways. *J Biol Chem* 268, 17830-17836

Urbach J, Rando RR (1994) Isomerization of all-*trans*-retinoic acid to 9-*cis*-retinoic acid. *Biochem J* 299, 459-465

Wind JC, Born W, Rijnsent A, Boer P, Fischer JA (1993) Stimulation of calcitonin/CGRP-I and CGRP-II gene expression by dibutyryl cAMP in a human medullary thyroid carcinoma (TT) cell line. *Mol Cell Endocrinol* 92, 25-31