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► **To cite this version:**

Hélène Roumes, Julie Brossaud, Aloïs Lemelletier, Marie-Pierre Moisan, Véronique Pallet, et al.. Retinoids and glucocorticoids have opposite effects on actin cytoskeleton rearrangement in hippocampal HT22 cells. *International Journal of Biochemistry and Cell Biology*, 2016, 71, pp.102-110. 10.1016/j.biocel.2015.12.014 . hal-04631897

**HAL Id: hal-04631897**

**<https://hal.science/hal-04631897v1>**

Submitted on 2 Jul 2024

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# The International Journal of Biochemistry & Cell Biology

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## Retinoids and glucocorticoids have opposite effects on actin cytoskeleton rearrangement in hippocampal HT22 cells



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### ARTICLE INFO

#### Article history:

Received 17 September 2015

Received in revised form

18 December 2015

Accepted 28 December 2015

Available online 31 December 2015

#### Keywords:

Retinoic acid

Glucocorticoid

Actin cytoskeleton

Hippocampus

### ABSTRACT

A chronic excess of glucocorticoids elicits deleterious effects in the hippocampus. Conversely, retinoic acid plays a major role in aging brain plasticity. As synaptic plasticity depends on mechanisms related to cell morphology, we investigated the involvement of retinoic acid and glucocorticoids in the remodelling of the HT22 neurons actin cytoskeleton.

Cells exhibited a significantly more elongated shape with retinoic acid and a rounder shape with dexamethasone; retinoic acid reversed the effects of dexamethasone.

Actin expression and abundance were unchanged by retinoic acid or dexamethasone but F-actin organization was dramatically modified. Indeed, retinoic acid and dexamethasone increased ( $70 \pm 7\%$  and  $176 \pm 5\%$ ) cortical actin while retinoic acid suppressed the effect of dexamethasone ( $90 \pm 6\%$ ). Retinoic acid decreased ( $-22 \pm 9\%$ ) and dexamethasone increased ( $134 \pm 16\%$ ) actin stress fibres. Retinoic acid also suppressed the effect of dexamethasone ( $-21 \pm 7\%$ ).

Spectrin is a key protein in the actin network remodelling. Its abundance was decreased by retinoic acid and increased by dexamethasone ( $-21 \pm 11\%$  and  $52 \pm 10\%$ ). However, retinoic acid did not modify the effect of dexamethasone ( $48 \pm 7\%$ ). Calpain activity on spectrin was increased by retinoic acid and decreased by dexamethasone ( $26 \pm 14\%$  and  $-57 \pm 5\%$ ); retinoic acid mildly but significantly modified the effect of dexamethasone ( $-44 \pm 7\%$ ). The calpain inhibitor calpeptin suppressed the effects of retinoic acid and dexamethasone on cell shape and actin stress fibres remodelling but did not modify the effects on cortical actin.

Retinoic acid and dexamethasone have a dramatic but mainly opposite effect on actin cytoskeleton remodelling. These effects originate, at least partly, from calpain activity.

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### 1. Introduction

Retinoic acid (RA, the main vitamin A metabolite) apart from playing a major role in brain development (Maden et al., 1998), is critically implicated in adult brain because of its involvement in cellular and synaptic plasticity (Chen et al., 2014; Lane and Bailey, 2005; McCaffery et al., 2006; Mey and McCaffery, 2004). Notably, it has been shown that the decreased retinoid signalling with age correlates with cognitive alterations (Bonhomme et al., 2014a; Etchamendy et al., 2001; Mingaud et al., 2008). By contrast,

aging is marked by an increased signalling of the glucocorticoid (GC) pathway (McEwen, 2007; Mohler et al., 2011; Yau et al., 2007). In humans and animals, this is correlated with hippocampus-dependent memory impairment (Lupien et al., 2009; Yau et al., 2007). In mice, GCs affect synaptic potentiation (Alfarez et al., 2002) and impair synaptic plasticity (Krugers et al., 2006) while RA seems to counteract some of the deleterious cognitive effects of GCs (Bonhomme et al., 2014a, 2014b; Touyarot et al., 2013). RA and GCs both act, mainly but not exclusively, through dimers of nuclear receptors (RAR and RXR, GR, MR) and RA and GC-response elements (RARE and GRE) (Bamberger et al., 1996; Piskunov et al., 2014). Interestingly, RA and GR signalling pathways may interact. For instance, RA potentiates GC-induced thymocytes apoptosis (Toth et al., 2011) and reduces GC sensitivity of skeletal muscle (Aubry and Odermatt, 2009) while dexamethasone (Dex) a GR-specific agonist enhances the RA-dependent increase of RAR 

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expression in hepatocytes (Yamaguchi et al., 1999). More recently, we have shown an interaction between these two pathways in the hippocampal HT22 cell line (Brossaud et al., 2013). Of note, this interaction targets the transcription and secretion of the neurotrophin brain-derived neurotrophic factor (BDNF).

Brain aging is marked by a decline of cognitive functions apparently related to a decreased neuronal plasticity, the basis of learning and memory process. Previous studies have shown that stress is associated with reduced dendritic branching and decreased spine density (Liston and Gan, 2011; McEwen et al., 1999). By contrast, other studies have shown that hippocampal neurons spine formation is induced by RA (Chen and Napoli, 2008) and by BDNF, a major regulator of synaptic plasticity of adult synapses (Bramham and Messaoudi, 2005; Magarinos et al., 2011). RA increases, whereas Dex reduces, the expression and abundance of the BDNF (Brossaud et al., 2013) whereas other factors contribute to normal brain function and plasticity. For instance, physiological levels of reactive oxygen and nitrogen species (ROS and RNS, respectively) contribute to synaptic plasticity and memory consolidation (Munnamalai and Suter, 2009; Wilson and Gonzalez-Billault, 2015). For various reasons the brain is particularly sensible to oxidation and any modification of the redox balance may have consequences on physiological responses. It appears that both retinoic acid and GCs interact with the redox balance particularly in neurons (da Frota Junior et al., 2011; Guleria et al., 2006; Spiers et al., 2014).

It can be argued that the molecular and cellular mechanisms underlying learning and memory are an adaptation of the mechanisms used by all cells to regulate cell motility (Baudry and Bi, 2013) and that cell motility involves cellular cytoskeleton remodelling. The cytoskeleton is composed of three major elements: microtubules, intermediate filaments and actin microfilaments. The latter are formed by polymerization of globular actin (G-actin) into filamentous actin (F-actin). The redox balance is one component of this cytoskeleton organisation. ROS target actin directly through the semaphorin signalling and ROS/RNS may act by opening calcium channels activating calcium-dependent processes (Munnamalai and Suter, 2009; Tiago et al., 2011; Wilson and Gonzalez-Billault, 2015). BDNF is another player where its effects on neurons involve at least two pathways: (i) regulation of the expression of genes involved in cellular plasticity such as activity-regulated cytoskeleton-associated protein (Arc) and Ca<sup>2+</sup>/calmodulin-dependent protein kinase II (CaMKII) (Bramham and Messaoudi, 2005; Larsen et al., 2007; Rao et al., 2006; Soule et al., 2006), and (ii) modifications of cytoskeleton organization (Gehler et al., 2004; Rex et al., 2007). Part of the F-actin cytoskeleton regulation depends on the activation of calcium-dependent endoproteases and the calpains (Baudry and Bi, 2013; Sato and Kawashima, 2001). The activity of calpains is mainly regulated by calpastatin which is their specific endogenous inhibitor.

On the basis of our recent results related to the different effects of RA and Dex treatments on hippocampal cells (Brossaud et al., 2013) we explored some pathways of neuroplasticity upon administration of retinoids and glucocorticoids pathway agonists. We then investigated the influence of RA and GC in the hippocampal HT22 cell line on (i) the expression of CaMKII and Arc genes and (ii) F-actin cytoskeleton organization including the implication of calpains.

## 2. Materials and methods

### 2.1. Cell cultures

Cell cultures were performed as described previously (Brossaud et al., 2013). HT22 cells were grown in a 5% CO<sub>2</sub> atmosphere at 37 °C in DMEM (Dubecco's modified Eagle medium, Life Technologies, Van Allen Way Carlsbad, CA, USA) with pyruvate supplemented

with 10% foetal bovine serum (FBS, Life Technologies) and 1% streptomycin sulfate/phenoxypenicilinic acid. Before all experiments, the cells were cultured for 48 h in DMEM supplemented with 0.1% FBS. For imaging experiments the cells were cultured on glass coverslips pretreated with gelatine.

### 2.2. Cells treatments

Treatments consisted of all-trans RA (final concentration 10<sup>-6</sup> M), Dex (final concentration 10<sup>-6</sup> M), or RA/Dex (final concentrations 10<sup>-6</sup> for both). RA and Dex stock solutions (Sigma–Aldrich, St Louis, MO, USA; 17.5 and 10 mM, respectively) were diluted into ethanol:dimethylsulfoxide (50:50) (VWR International, West Chester, PA, USA) added as vehicle in control. The culture medium was replaced after 2 d and the treatment duration was 4 d (Brossaud et al., 2013).

The chemical calpain inhibitor calpeptin (Z-Leu-Nle-CHO) was obtained from Merck (Nottingham, UK) and used at final concentration of 50 μM. Calpeptin was added 4 h before experiments to culture medium (Roumes et al., 2010).

### 2.3. Morphological characterization and actin cytoskeleton organization analysis

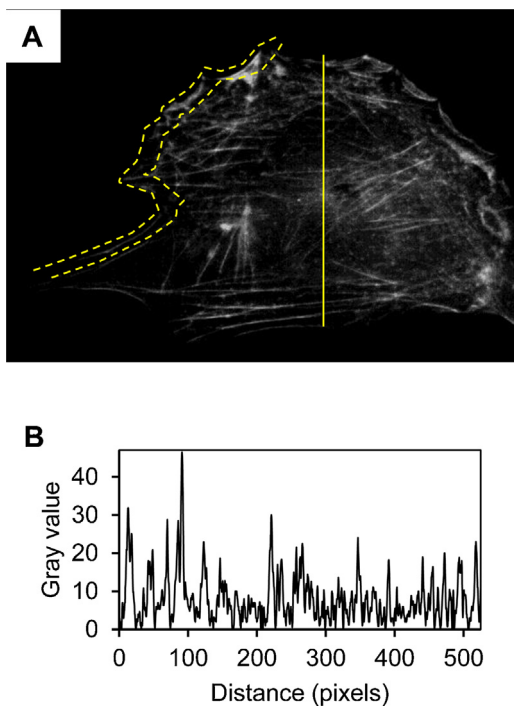
After 4 days of treatment (seeding density: 7500 cells/cm<sup>2</sup>), the cells were fixed (paraformaldehyde 4%, 15 min) and their morphologies were observed in phase contrast. Images were analysed using ImageJ software. Cell areas, nuclear area, nucleus/cytoplasm ratio and the degree of circularity were determined. For the latter we used a feature of ImageJ (circularity = 4π × Area/Perimeter<sup>2</sup>) where, a perfect circle would have circularity of 1.

For actin quantification, F-actin was stained as previously described (Roumes et al., 2010). After fixation the cells were permeabilized (Triton X-100 1%, 3 min) while F-actin was stained with phalloidin-fluorescein-5-isothiocyanate (FITC, 0.5 μM) and the nucleus with 4'-6-diamino-2-phenylindole (DAPI, 1.5 μg/ml) for 40 min. After three washings, the cells were observed using an epifluorescence microscope (Leica AF DMI6000) or a confocal microscope (Olympus, Fluoview 300). Images were taken at 40x magnification and analysed using ImageJ software. Tiff images were converted to 8-bit grayscale, calibrated in ImageJ to optical density (OD) values and the background was subtracted. The threshold on the images was adjusted until actin fluorescence was excluded from the threshold limits (Peacock et al., 2007). The following measurements were made to quantify F-actin: F-actin area, cortical actin along cell borders, stress fibres that traverse the cell and stress fibres/cell area ratio. Cortical actin was detected and analysed using the freehand selection tool. For stress fibre quantification, a line selection perpendicular to the stress fibres orientation was made (Fig. 1A). The ImageJ plot profile was used to obtain the stress fibre density (Fig. 1B).

For each imaging experiment conducted at least three times, more than 20 cells per condition were examined.

### 2.4. Real-time PCR analyses

Real-time PCR analyses were performed using the LightCycler<sup>®</sup> 480II system (Roche) as described previously (Brossaud et al., 2013). Specific primers were as follows: GAACATCATCCCTGCATCCA and CCAGTGAGCTTCCCGTTCA for glyceraldehyde 3 phosphate dehydrogenase (GAPDH); GGCTGTATCCCCTCCATCG and CCAGTTGGTAACAATGCCATGT for actin; TCCTGCAGATTG-GTAAGTGC and GTGCAACCTTTCAGTCTC for Arc; ATCGG-GTGGGCTCTATGGCTTCTG and TGCACTTCCCTCTGCATGCACG for CaMKII. All these primers were generated from the respective nucleotide sequences obtained from the National Centre for



**Fig. 1.** Example of cortical actin and stress fibres quantification. Cortical actin was detected and analysed using the freehand selection tool ((A) dotted line). For stress fibre quantification, a line selection perpendicular to the stress fibre orientation was made ((A) solid line). The ImageJ plot profile was used to obtain the stress fibre density (B). AU arbitrary unit.

Biotechnology Information (NCBI) gene bank. The GAPDH house-keeping gene was used as reference gene for relative quantification. Quantification of the data was performed using LightCycler480 Relative Quantification software (version 1.5).

### 2.5. Calpain activity

Calpain activity was evaluated *via* the quantification of the calpain-specific spectrin cleavage products by Western blot (Dourdin et al., 2001). Protein extraction, electrophoresis, and transfer were performed as described previously (Mingam et al., 2008). The membranes were incubated with specific antibodies against alpha-II spectrin (rabbit anti-alpha-II spectrin, Abcam, ab75755, 1:2000) (Li et al., 2013) and against actin (rabbit anti-actin, Sigma-Aldrich, A2066, 1:2500). The second horseradish peroxidase-conjugated antibody (donkey anti-rabbit, Jackson Immunoresearch, 1:5000) was used for detection. The blots were developed using Western Lighting Chemiluminescence Reagent Plus (PerkinElmer). GAPDH was used as a housekeeping gene

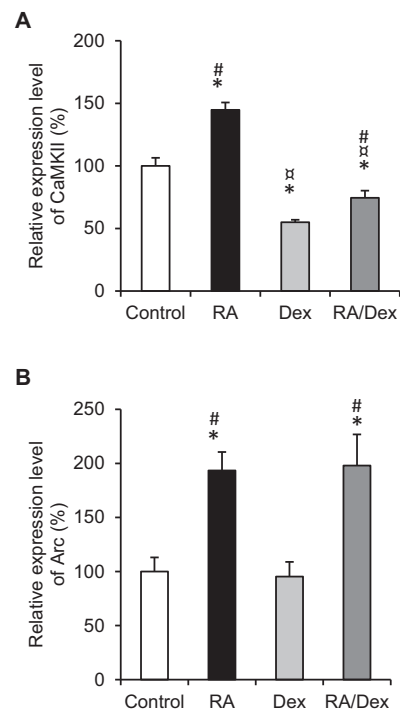
### 2.6. Data analysis

Unless otherwise indicated, all data were expressed as mean  $\pm$  SEM, calculated for at least three independent experiments. The statistical significance of the differences between multiple groups was determined using non-parametric Kruskal–Wallis' test. When the statistic was associated with a  $p < 0.05$  probability, inter-group comparisons were conducted using Mann-Whitney *U*-test.

## 3. Results

### 3.1. RA and Dex alter CaMKII and Arc mRNA expression

We quantified the expression of two important plasticity genes (Larsen et al., 2007; Mingaud et al., 2008; Sato and Kawashima,



**Fig. 2.** Consequences of retinoic acid (RA  $10^{-6}$  M) and/or glucocorticoid (Dex  $10^{-6}$  M) pathway activation in plasticity BDNF-dependant gene expression. Relative quantification of CaMKII (A) and Arc (B) mRNA by real-time RT-PCR. Mean  $\pm$  SEM from at least three independent experiments. (\*) Significantly different from the control, (\*\*) from RA-treated cells, and (#) from Dex-treated cells ( $p < 0.05$ ).

2001; Soule et al., 2006): CaMKII and Arc mRNA after RA and/or Dex exposure (Fig. 2).

RA significantly increased CaMKII mRNA expression compared to control (increase  $+44.8 \pm 5.8\%$ ) (Fig. 2A). Conversely, Dex significantly decreased CaMKII mRNA expression (decrease  $-45.1 \pm 1.9\%$ ). RA significantly reduced the effect of Dex (decrease  $-25.5 \pm 5.7\%$ ).

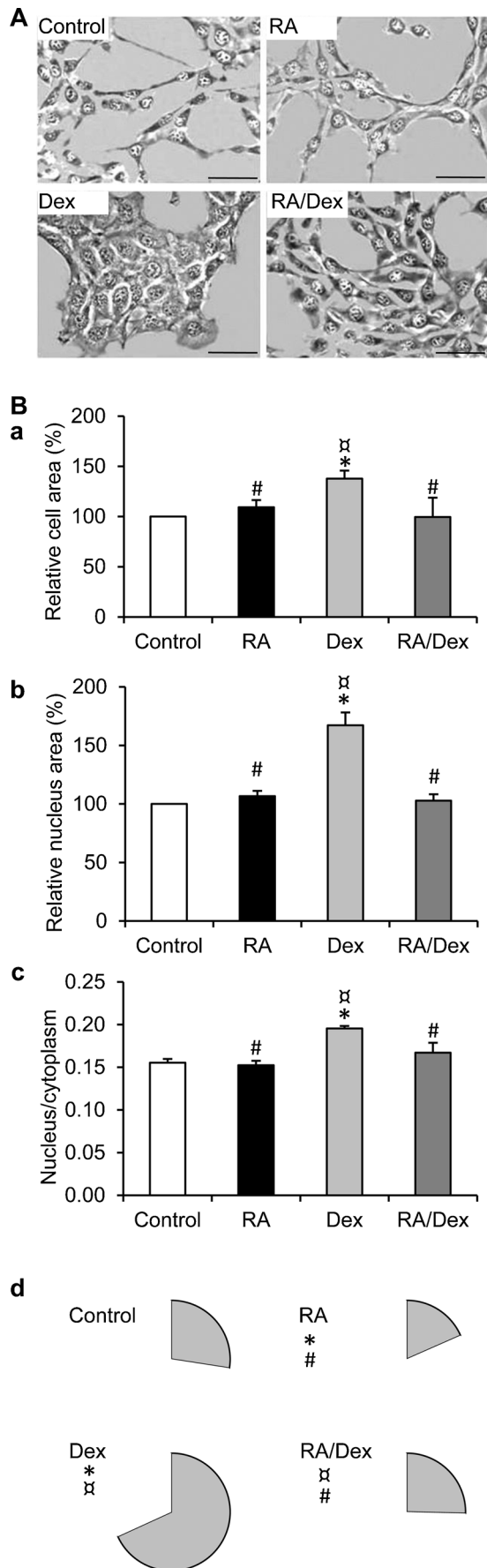
RA significantly increased Arc mRNA expression compared to control (increase  $+93.5 \pm 16.9\%$ ) (Fig. 2B). Dex did not modify Arc mRNA expression. RA added to Dex achieved the same level as RA alone (increase  $+98.03 \pm 28.7\%$ ).

### 3.2. Activation of GC signalling pathways leads to morphological changes in hippocampal cell line, and is modulated by RA treatment

We investigated whether activation of RA and GC signalling pathways could modify hippocampal HT22 cell shape. Indeed, RA and/or Dex qualitatively affected cell morphology (Fig. 3A). Control, RA- and RA/Dex-treated cells appeared elongated with membrane protrusions. Dex-treated cells exhibited a round shape, a reduced cytoplasm area and a large nucleus.

The area of control and RA-treated cells were similar. Conversely, the area of Dex-treated cells was significantly larger than the control (Fig. 3Ba). RA reduced the area of Dex-treated cells to control levels. The area of the nucleus of control and RA-treated cells were similar. Conversely, the area of the nucleus of Dex-treated cells was significantly larger than control (increase  $+67.2 \pm 11.0\%$ ) (Fig. 3Bb). RA reduced the area of the nucleus of Dex-treated cells to control levels.

RA alone did not affect the nucleus/cytoplasm ratio compared to control (Fig. 3Bc). This ratio was significantly increased by Dex. RA reduced the ratio of Dex-treated cells although not down to control levels.



**Fig. 3.** Consequences of retinoic acid (RA  $10^{-6}$  M) and/or glucocorticoid (Dex  $10^{-6}$  M) pathway activation in morphological characteristics of HT22 cells. Phase

We thus focused on the circularity of the cells (see material and methods). Compared to control cells, RA-treated cells exhibited a more elongated shape (Fig. 3Bd). Dex-treated cells exhibited a rounder shape. RA/Dex treated cells have the same global morphology as control cells.

### 3.3. Activation of RA and GC signalling pathways leads to actin cytoskeleton reorganization

Cell morphology is closely related to the regulation of actin architectural frame. Therefore, we studied actin mRNA expression and protein abundance and organization under the RA and/or Dex treatments.

Quantification of actin expression and abundance by RT-qPCR and Western blots, respectively did not show any significant effect of the treatments (data not shown).

Polymerized F-actin was stained with phalloidin-FITC (Fig. 4A). RA led to a minor but significant increase of total F-actin (increase +14.0 ± 3.8%) (Fig. 5A). A large increase of total F-actin was induced by Dex (increase +100.2 ± 10.2%). RA combined to Dex reduced total F-actin to basal level (decrease -4.3 ± 19.6%). Cortical actin and stress fibres were subsequently discriminated. RA mildly but significantly increased whereas Dex dramatically increased cortical actin. RA combined to Dex reduced the effect of Dex on cortical actin down to RA level (Fig. 5B). RA had no significant effect on stress fibres (Fig. 5C). Dex significantly increased actin stress fibres. RA combined to Dex reduced the effect of Dex on stress fibres down to basal level. The stress fibres/cell area ratio was calculated (Fig. 5D). RA significantly decreased whereas Dex significantly increased this ratio. RA combined to Dex reduced the ratio down to RA level.

To summarize, actin expression and abundance were unchanged by Dex and/or RA, and, conversely, F-actin organization was modified by both Dex and RA.

### 3.4. Activation of RA and GC signalling pathways alters spectrin abundance and modulates calpain activity

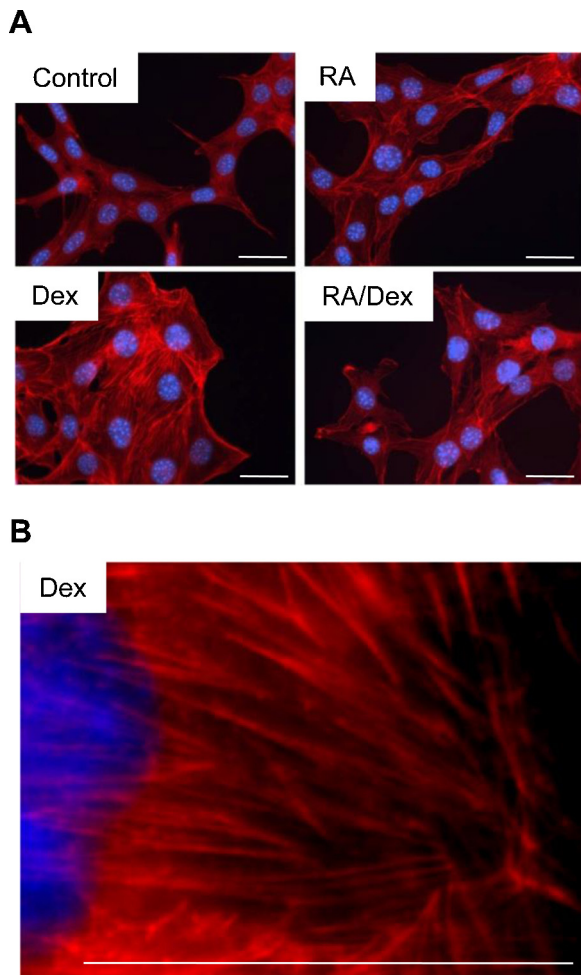
As the F-actin filaments are inter-connected by spectrin, the spectrin abundance was quantified (Fig. 6A and B). RA significantly decreased whereas Dex significantly increased spectrin levels. RA combined to Dex failed to modify Dex-induced spectrin increase.

As calpains cleaves spectrin molecules thus triggering the elongation of actin filaments (Baudry and Bi, 2013; McCaffery et al., 2006), we evaluated calpain activity by quantifying truncated spectrin (evaluation of the truncated/non-truncated spectrin ratio) (Fig. 6B). RA significantly increased whereas Dex treatments led to a decrease of calpain activity (RA +26.1 ± 14.0%; Dex -56.9 ± 4.7%). RA combined to Dex mildly reduced the effects of Dex alone.

### 3.5. Calpains are implicated in the RA- and Dex-induced cytoskeleton reorganization

To highlight the involvement of calpains in cell morphology and cytoskeleton rearrangement, we treated cells with a calpain

contrast images of control, RA and/or Dex treated cells (A). Quantitative parameters using ImageJ: Relative cell area (Ba), nuclear area (Bb) and nucleus/cytoplasm ratio (Bc) of control, RA and/or Dex treated cells. Evaluation of the circularity degree of cells (Bd) for each condition: a perfect circle with circularity of 1 would be represented by a complete disk; the small the circular sector the less circular the cells (i.e. more elongated): control  $0.27 \pm 0.01$ , RA  $0.18 \pm 0.01$ , Dex =  $0.68 \pm 0.02$ , RA/Dex =  $0.25 \pm 0.01$ . Mean ± SEM from at least three independent experiments. (\*) Significantly different from the control, (α) from RA-treated cells, and (#) from Dex-treated cells ( $p < 0.05$ ). The images are representative of at least three separated sets of culture. Scale bar: 50 μm.



**Fig. 4.** Consequences of retinoic acid (RA  $10^{-6}$  M) and/or glucocorticoid (Dex  $10^{-6}$  M) pathway activation in actin cytoskeleton organization of HT22 cells. F-actin organization of cells for each condition (A). F-actin was stained with phalloidin-FITC ( $0.5 \mu\text{M}$ ) and nucleus with DAPI ( $1.5 \mu\text{g/ml}$ ).

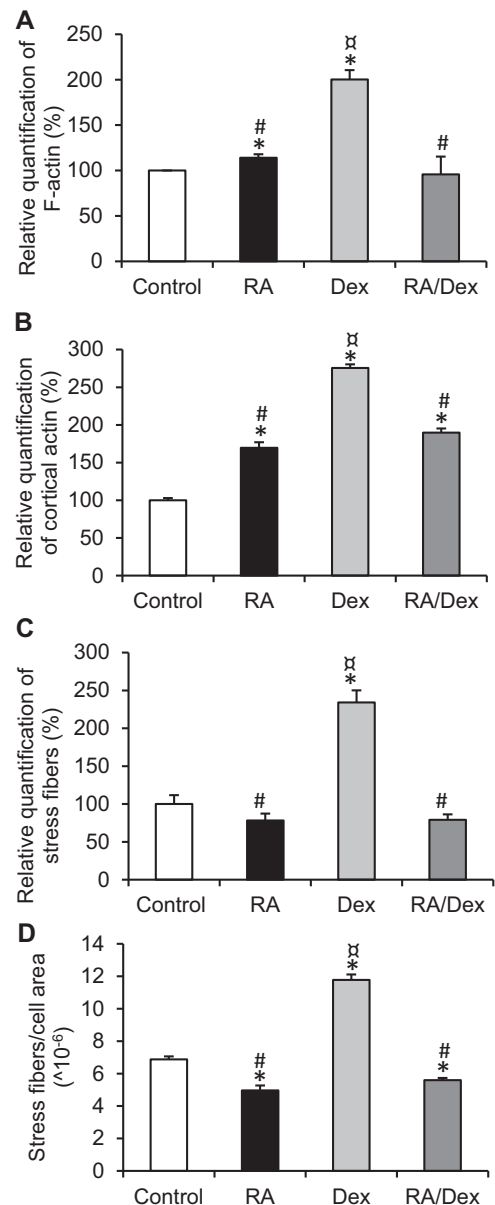
inhibitor, calpeptin. Calpeptin ( $50 \mu\text{M}$ , 4 h), eliminated the ability of RA and Dex to significantly modify cell area (Fig. 7A). Similarly, calpeptin eliminated the ability of RA and Dex to significantly modify circularity (Fig. 7B).

Calpeptin inhibited the effects of RA and/or Dex on total phalloidin-labelled F-actin (Fig. 8A). Similarly, after calpeptin treatment, RA and/or Dex did not modify total F-actin amounts (data not shown).

In presence of calpeptin, RA mildly but significantly increased cortical actin (increase  $+14.4 \pm 2.6\%$ ) and Dex dramatically increased cortical actin (increase  $+273.6 \pm 48.7\%$ ) (Fig. 8B). RA combined to Dex reduced the effects of Dex alone. Thus, the results are very similar to those obtained in the absence of calpeptin (Fig. 5B). Conversely, calpeptin inhibited the effects of RA and/or Dex on stress fibres as the stress fibres/cell area ratio was unchanged in any condition (Fig. 8C).

#### 4. Discussion

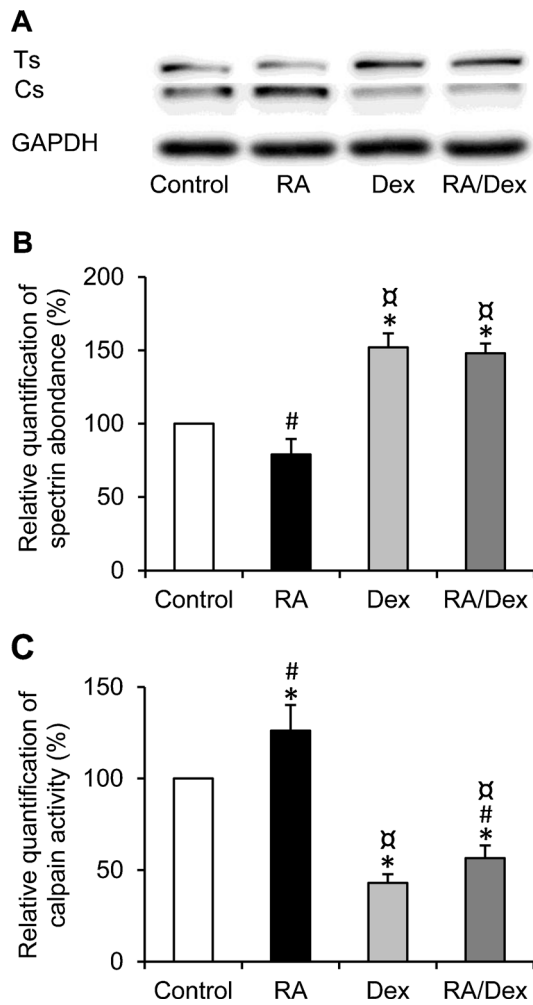
We investigated selected neuronal plasticity-related mechanisms that can be used to decipher interactions between retinoid and glucocorticoid signalling pathways. We used hippocampal cells from the HT22 line. Its origin the mouse hippocampus, interested us as a result of prior work on memory and aging in the mouse (Bonnet et al., 2008; Mingaud et al., 2008; Touyarot et al., 2013).



**Fig. 5.** Relative quantification of total F-actin (A), cortical actin (B) and stress fibres (C) of untreated and treated cells. Stress fibres/cell area ratio (D) for each condition. (\*) Significantly different from the control, (x) from RA-treated cells, and (#) from Dex-treated cells ( $p < 0.05$ ). The images are representative of at least three separated sets of culture. Scale bar:  $20 \mu\text{m}$ .

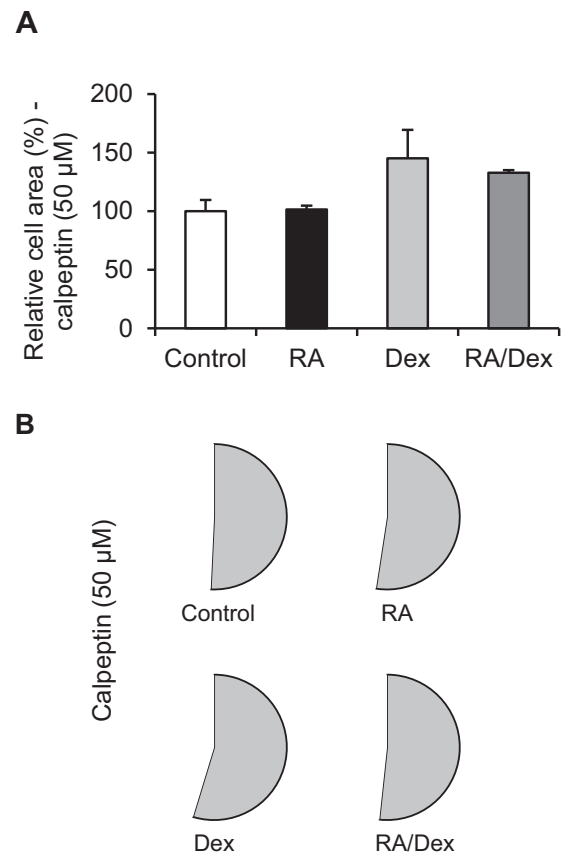
The current investigation was partly initiated because of existing arguments for neuronal plasticity to be an adaptation of the mechanisms used by the cells to regulate their motility (Baudry and Bi, 2013). In a previous paper, we showed some opposite effects of retinoids and glucocorticoids signalling on their own receptors and on BDNF and tissue-transglutaminase synthesis (Brossaud et al., 2013). Neuroplasticity implies modifications of synaptic strength through different mechanisms such as modifications of transcription and local translation, activation of protein kinases and calpain proteases, modification of actin filament assembly (Panja and Bramham, 2014). We then studied the effects of retinoids and glucocorticoids signalling on actin remodelling on HT22 neurons.

We investigated these effects on neuronal plasticity via the regulation of cytoskeleton organization (Gehler et al., 2004; Rex et al., 2007). Cell morphology changed dramatically in presence of RA or Dex. The activation of the retinoids pathway led to an



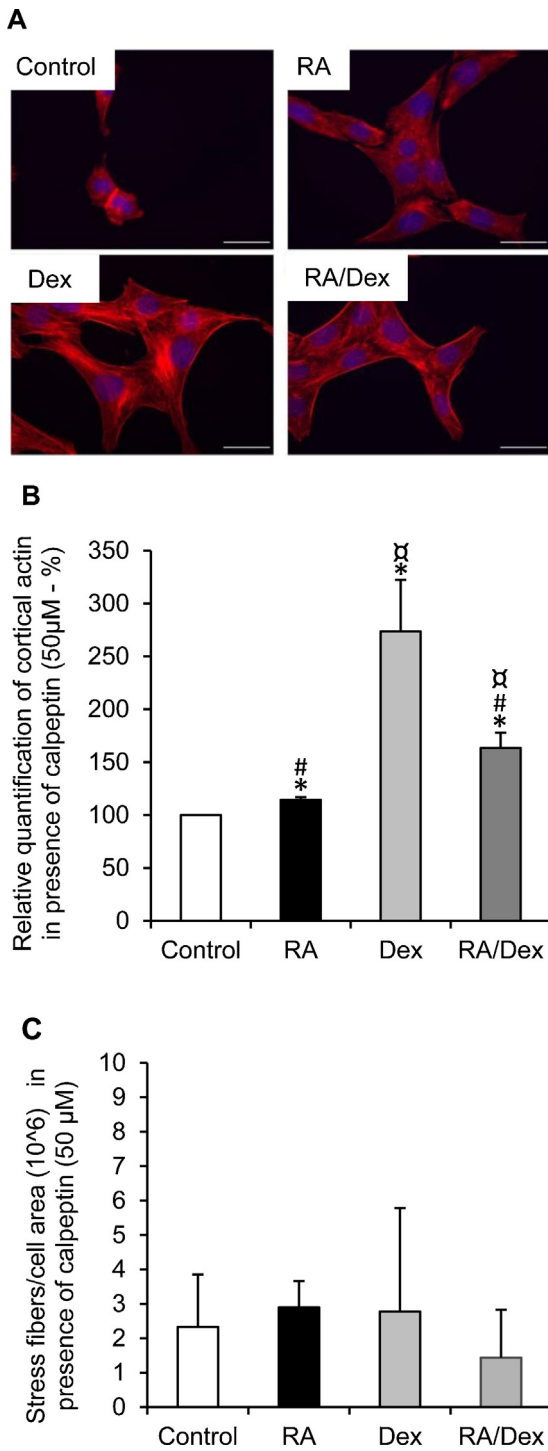
**Fig. 6.** Effects of RA ( $10^{-6}$  M) or Dex ( $10^{-6}$  M) treatments on spectrin abundance and calpain activity, both detected by Western blots. Western blot results for analysing calpain activity (A), Ts: total spectrin, Cs: cleaved spectrin, GAPDH: Glyceraldehyde 3-phosphate dehydrogenase (housekeeping gene). Relative quantification of spectrin abundance (B). Relative calpain activity (C). Mean  $\pm$  SEM from at least three independent experiments. (\*) Significantly different from the control, (#) from RA-treated cells, (#) from Dex-treated cells ( $p < 0.05$ ).

elongated shape presumably favouring the development of protrusions such as filipodia. Conversely, GC signalling morphed the cells toward a rounded shape limiting the development of protrusions. These morphological changes suggest the RA signalling enhances neuronal plasticity because of the stimulation of protrusion formations. Consequently, the actin cytoskeleton, responsible for cell morphology and cell movements (Lambrechts et al., 2004), was also investigated. The long-term (4 d) effects of RA and Dex were investigated. This was too long to allow the acquisition of rapid dynamic data using time-lapse imaging of actin polymerisation and depolymerisation using specific tracers of G and F actin (Tiago et al., 2011). The GC signalling was responsible of a major remodelling of actin cytoskeleton. This is consistent with previous demonstrations that Dex led to formation of stress fibres across the cell body, the adhesion assembly (Mayanagi et al., 2008), and to the formation of cross-linked actin networks (Clark et al., 1994). These Dex-induced actin structures across the cell body should not favour neural plasticity. By contrast, the RA signalling pathway activation led to elongated cells along with increases in cortical actin polymerization. It has been previously established that after a RA treatment  $\mu$ -calpain activity increased in cells that extended neuritis (Grynspan et al., 1997). Likewise, the expressions



**Fig. 7.** Consequences of calpain inhibition (calpeptin 50  $\mu$ M) in morphological characteristics of HT22 cells, treated or not with RA ( $10^{-6}$  M) or Dex ( $10^{-6}$  M). Relative cell area (A), evaluation of the circularity degree of cells (B) for each condition. Total circle represents "1": a perfect circle. The more shaded area represents a large portion of the disk, the more cells are rounded. Conversely, the more shaded area represents a small part of the disk, the more cells are elongated. Control  $0.51 \pm 0.02$ , RA  $0.55 \pm 0.02$ , Dex  $0.52 \pm 0.02$ , RA/Dex  $0.52 \pm 0.02$ . Mean  $\pm$  SEM from at least three independent experiments.

of  $\mu$ - and m-calpain were increased during RA-induced differentiation (Truckenmiller et al., 2001). In agreement, our results show an increased calpain activity under RA treatment correlated with reduced amounts of stress fibres. Such a phenomenon has been described in vSrc-transformed cells in which calpain activity stimulates the loss of actin stress fibres (Carragher et al., 2001). This process may be responsible for increased cell motility (Perrin et al., 2006) and possibly potentiation of synaptic plasticity (Baudry and Bi, 2013). On the other hand, the effects of GC stimulation pathways on calpain activity are more controversial. GC pathway may activate (Hayashi et al., 2000; Suwanjang et al., 2013) or inhibit calpain activity (Baquedano et al., 2011; Maes et al., 2008; Sur et al., 2005). In our model, the GC-induced decrease of calpain activity could explain the observed cytoskeleton reorganization. HT22 cells became rounder when calpain activity was inhibited regardless of their treatment. This has previously been observed after calpeptin treatment (Roumes et al., 2010) or calpastatin overexpression (Potter et al., 1998). In our model, calpain inhibition disorganized the central actin cytoskeleton inhibiting the effects of RA or Dex treatments. The effects of calpeptin on cortical actin level are in agreement with those obtained in muscle cells (Roumes et al., 2010) or cells overexpressing calpastatin (Dedieu et al., 2004). The persistence of cortical actin organization coupled with the disorganization of central fibres could be explained by the existence of two different pathway involved in stress fibre organization: one regulates peripheral stress fibres, depending on calcium and calmodulin



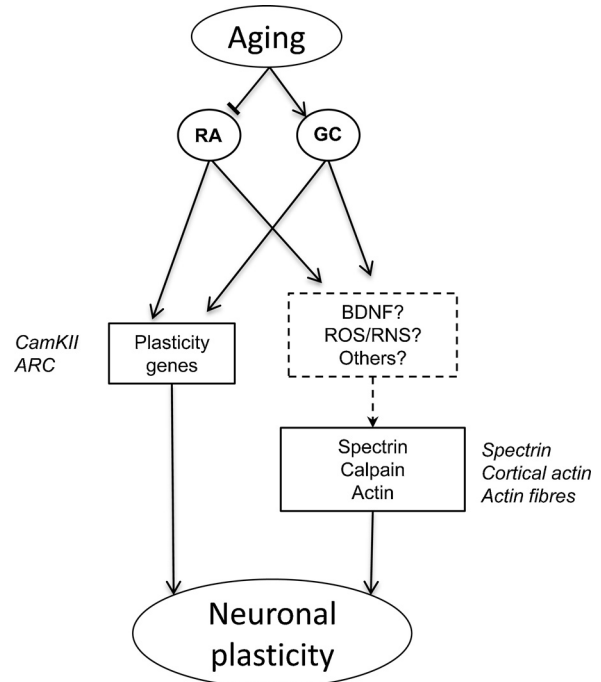
**Fig. 8.** Consequences of calpain inhibition (calpeptin 50 μM) in actin cytoskeleton organization of HT22 cells, treated or not with RA (10<sup>-6</sup> M) or Dex (10<sup>-6</sup> M). F-actin organization of cells for each condition (A). Relative quantification of cortical actin (B). Stress fibres/cell area ratio (C) for each condition. (\*) Significantly different from the control, (α) from RA-treated cells, and (#) from Dex-treated cells (*p* < 0.05). The images are representative of at least three separated sets of culture. Scale bar: 20 μm.

while the other involves Rho and Rho-kinase (Kato et al., 2007). Interestingly, CaMKII may also be involved in cellular remodelling. Indeed, CaMKII binds and cross-links F-actin while it regulates the actin cytoskeleton (Hell, 2014, Okamoto et al., 2009). Therefore, CamKIIβ facilitates bundling of actin filaments and dendritic spine enlargement in a kinase-independent manner. Furthermore, the cleavage of the CaMKII by calpains could lead to a loss of the

CaMKII auto-inhibitory domain and thus cause an irreversible activation (Hajimohammadreza et al., 1997). CaMKII is a critical kinase involved in neuroplasticity and long-term potentiation (Baudry et al., 2014). RA can induce neurite extensions that are coincident with a large increase in CaMKII-II activity (Johnson et al., 2000). In our model, RA increases (while Dex decreases) CaMKII mRNA expression. This up-regulation may be explained by the presence of two RARE on the CaMKII gene promoter (Wang et al., 2008). The effect of RA-induced CaMKII mRNA expression was reduced by Dex. This coincides with several studies including a recent one by Tongjaroenbuangam et al. that shows a decline of hippocampal CaMKII in Dex-treated mice (Tongjaroenbuangam et al., 2013). CaMKII can be activated both by BDNF (Blanquet et al., 2003) and by ROS-induced intracellular calcium rises via membrane calcium channels (Tiago et al., 2011).

We also investigated the transcription of Arc, a plasticity-associated transcript (Nakayama et al., 2015). Arc mRNA is translocated into dendritic spines and is locally translated where the Arc protein is involved in synaptic remodelling. In our model, Arc transcription is regulated by the retinoid pathway but not by the GC pathway. To our knowledge this has not been reported until now.

In this study, RA reversed the effects of Dex totally on cell morphology but incompletely on calpain activity. This may mean that the effects of RA are not mediated through calpain only or that the partial effects of RA on Dex-modulated calpain activity are sufficient to fully reverse the effects of Dex on cell morphology. We show an interaction between the RA and Dex signalling pathways both on actin cytoskeleton and on plasticity gene expression (Fig. 9) as previously exhibited on other genes including BDNF and tissue-transglutaminase (Brossaud et al., 2013; Katsuki et al., 2009; Kurauchi et al., 2011). The molecular steps between RA and Dex, and actin remodelling have to be investigated. Both autocrine



**Fig. 9.** Aging decreases retinoic acid (RA) signalling and, conversely, accumulates glucocorticoids (GC) deleterious effects. RA and GC have different effects and often-times opposite on some neuroplasticity genes. RA and GC have also opposite effects on actin fibre remodelling. The precise, yet unknown, mechanism of action may involve reactive oxygen/nitrogen species and calcium entry, autocrine actions of the neurotrophin BDNF, or other undefined mechanisms. RA and GC target the actin network through actions on calpain and spectrin. Both transcription regulation and actin network remodelling act on neuronal plasticity.



BDNF and intracellular tissue-transglutaminase may be involved as they are implicated in spine mobility (Horch et al., 1999), new thin spine production (Ji et al., 2010), calpain activity (Zadran et al., 2010) and cell migration and invasion (Joshi et al., 2006). Alternatively, RA- and Dex-induced alterations of the redox balance may also be involved *via* calcium entry and calpain activation (da Frota Junior et al., 2011; Guleria et al., 2006; Spiers et al., 2014; Wilson and Gonzalez-Billault, 2015). Our research shows that pharmacological targeting common elements *e.g.* calpains, may be useful to counteract some of the deleterious effects of chronic glucocorticoid exposure and of the cognitive impairment due to aging.

### Conflict of interest

The authors declare no competing financial interests.

### Acknowledgments

The authors wish to thank Dr E. Maronde for an earlier gift of HT22 cells. The authors also wish to thank LCol R. Poisson for reviewing and improving the text.

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