Biochemical and Biophysical Research Communications 394 (2010) 233–239

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Cardiac glycosides potently inhibit C-reactive protein synthesis in human hepatocytes

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ARTICLE INFO

Article history:
Received 26 February 2010
Available online 3 March 2010

Keywords:
C-reactive protein
Cardiac glycosides
Cardiovascular disease
Inhibitors
Hepatocytes

ABSTRACT

Elevated plasma levels of C-reactive protein (CRP), the prototype acute-phase protein (APP), are predictive for future cardiovascular events. Controversial evidence suggests that CRP may play a causal role in cardiovascular disease. CRP synthesis inhibition is a potential approach for reducing cardiovascular mortality.

We show here that endogenous and plant-derived inhibitors of the Na^+/K^+-ATPase, i.e. the cardiac glycosides ouabain and digitoxin, inhibit IL-1β- and IL-6-induced APP expression in human hepatoma cells and primary human hepatocytes (PHH) at nanomolar concentrations. Inhibition is demonstrated on transcriptional and on protein level. The molecular target of cardiac glycosides, i.e. the α1 subunit of the Na^+/K^+-ATPase, is strongly expressed in human hepatocytes. Inhibition of APP synthesis correlates with the potency of cardiac glycosides at the Na^+/K^+-ATPase. The trigger for APP expression inhibition is an increase in intracellular calcium since the calcium ionophore calcimycin is also active. Qualified specificity of oubain for hepatocellular APP synthesis inhibition is demonstrated by lack of effectivity on IL-1β-induced IL-6 release from primary human coronary artery smooth muscle cells.

The inhibitory activity of cardiac glycosides on CRP expression may have important implications for the treatment of cardiovascular disease. Cardiac glycosides may be used for CRP synthesis inhibition in the future.

1. Introduction

The mammalian liver releases a set of proteins in response to systemic inflammation. Some of these so called acute-phase proteins [APPs, e.g. C-reactive protein (CRP), serum amyloid A (SAA), α1 acid glycoprotein (AGP), and fibrinogen] are used as clinical correlates for inflammation [1]. Elevated plasma levels of CRP are predictive for future cardiovascular events [2], and there is indeed evidence that CRP plays a direct role in the process of atherogenesis [3] by activating the complement system and activation of macrophages via Fcγ-receptors [4,5]. Both, the role of CRP as a risk marker and as a causal factor are currently intensively discussed [6,7]. Specific CRP inhibition may be regarded as the ultimate proof or disproof for a causal role of CRP in cardiovascular disease and its clinical relevance [3,7].

The major stimuli for release of CRP and other APPs by hepatocytes are the cytokines interleukin-1β (IL-1β) and interleukin-6 (IL-6) [1]. It is well documented that IL-1β and IL-6 synergistically act on CRP expression [8,9]. Recent studies suggest that the signal transducer and activator of transcription 3 (STAT-3), nuclear factor κB (NFκB), members of the C/EBP family (CCAAT box/Enhancer-Binding Protein) and hepatocyte nuclear factor 1 (HNF-1) are the main transcription factors involved in the transcriptional regulation of APP expression [9].

Many cardiovascular drugs are also able to modify the plasma levels of CRP (for review see: Ref. [10]). Especially the CRP-lowering effect of statins at least contributes to the favorable outcome of statin therapy even in apparently healthy individuals with normal low density lipoprotein (LDL) cholesterol [11]. The results from JUPITER (Justification for the Use of Statins in Prevention: an Intervention Trial Evaluating Rosuvastatin) trial demonstrated that cardiovascular events and mortality risk could be significantly lowered by treating patients who have normal LDL-C but elevated high-sensitivity CRP (hsCRP) with high-dose statin therapy [11].

However, so far there are only very few examples in the literature describing a systematic approach for identifying either specific compounds or cellular target molecules which interfere with human APP expression [12]. Such approaches are difficult since

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doi:10.1016/j.bbrc.2010.02.177

CRP protein synthesis seems to be restricted to primary human hepatocytes (PHH), which are limited due to ethical reasons and cell culture problems. On the other hand, widely available human hepatoma cell lines such as HepG2 and HUH-7 enable investigations of signal transductions from endogenously expressed receptors and enzymes to transfected APP promoter regions fused to convenient reporter genes [12]. To this end, we developed stable human hepatoma cell lines for screening libraries of drugs with known cellular targets upon their abilities to interfere with the IL-1β-and IL-6-induced APP expression. This approach revealed a surprising result. We identified endogenous and plant-derived inhibitors of the Na^+/K^+-ATPase, which are in therapeutical use since decades, as potent blockers of the IL-1β- and IL-6-induced C-reactive protein expression in human hepatocytes at nanomolar concentrations.

2. Methods

2.1. Materials

Primary human hepatocytes (PHHs) and hepatocyte culture medium (HCM) were obtained from XenoTech/Tebu-Bio. Three different lot numbers, #455 (female Caucasian, age 37 years), #700 (male Hispanic, 32 years) and #768 (female caucasian, 63 years) were used.

Primary human coronary artery smooth muscle cells (CASMCs) and smooth muscle cell growth media (Sm-GM) were obtained from Lonza.

The human hepatoma cell line HUH-7 was purchased from American Type Culture Collection. Recombinant human IL-1β and human IL-6 were from R&D Systems.

All compounds and a library of 1280 pharmacologically active compounds (LOPAC^{1280}), each specifically acting on an enzyme, receptor or ion channel were purchased from Sigma–Aldrich.

2.2. Quantitative real-time RT-PCR analysis

Quantitative TaqMan analysis was performed using the Applied Biosystems PRISM 7900 sequence detection system (Applied Biosystems, Foster City, CA). Human tissue RNA probes were obtained from Ambion, Inc. (Austin, TX), BioChain Institute, Inc. (Hayward, CA), Clontech Laboratories (Mountain View, CA), and 3H Biomedical AB (Uppsala, Sweden). Cell line RNAs were isolated using TRIzol Reagent (Invitrogen, Carlsbad, CA). The total RNAs were digested with DNase I and reversely transcribed using random hexamers. Selective probes were designed and comparable probe efficencies were assured by titration of genomic DNA. Normalization was performed using L32 as control, and relative expression was calculated using the formula: relative expression = $2^{(20 - (Ct(probe) - Ct(L32)))}$. The resulting expression is given in arbitrary units. The primers and fluorescent probes used are shown in Table 1.

2.3. Cell culture

PHHs and coronary artery smooth muscle cells (CASMCs) were maintained in HCM and Sm-GM. Human hepatoma HUH-7 cells were cultured in RPMI 1640 (Gibco) supplemented with 10% fetal calf serum (FCS, Gibco), 1% Hepes (Gibco), 1% non-essential amino acids (NEAA, Gibco), and 1% penicillin/streptomycin (Gibco).

For ligand treatment, stably transfected HUH cells were seeded in 96 well plates (5000 cells/well) or 384 well plates (4000 cells/well) in RPMI 1640 Medium (Gibco) containing 2% charcoal-treated FCS (Hyclone), 1% Hepes, 1% non-essential amino acids (NEAA, Gibco), 1% penicillin/streptomycin (Gibco), and 1 mg/ml G418. Cells were treated with compounds followed by 1 ng/ml IL-1β or 10 ng/ml IL-6 in serum-free medium 24 h (96 well plates) or 48 h (384 well plates) later. Luciferase activity was determined 24 h later using a Luciferase Reporter Assay System (Promega). The GraphPad Prism Software (version 3.02, GraphPad Software Inc., San Diego, CA) was used for curve fitting and calculation of the half-maximal effective (EC_{50}) or inhibitory concentration (IC_{50}), respectively.

Table 1
Primers and fluorescent probes[a].

ATP1A1	Forward primer: 5′-CACACAGCCTTCTTCGTCAG-3′
	Probe: 5′-(FAM) CAGTGGGCCGACTTGGTCATCTGT(TAMRA)-3′
	Reverse primer: 5′-ACCGAATTCCTCCTGGTCTT-3′
ATP1A2	Forward primer: 5′-GAAGGTGGTGGAGTTCACG-3′
	Probe: 5′-(FAM)ATTCTTTGCCAGCATCGTGGTGGT(TAMRA)-3′
	Reverse primer: 5′-GATGATGAGGTCAGCCCACT-3′
ATP1A3	Forward primer: 5′-TGTGATCCTGGCAGAAAATG-3′
	Probe: 5′-(FAM)CTTCTTGCCCGGCAACCTGGT(TAMRA)-3′
	Reverse primer: 5′-GTGCGGTCATCCCAGTTC-3′
ATP1A4	Forward primer: 5′-TTAGGCCTGTTGATCTGCTG-3′
	Probe: 5′-(FAM)CATCCGCCTCCACTGGGAAGATAAA(TAMRA)-3′
	Reverse primer: 5′-CACTGCTGTCCGTAGCTGTC-3′
L32	Forward primer: 5′-AAGTTCATCCGGCACCAGTC-3′
	Probe: 5′-(FAM)CCCAGAGGCATTGACAACAGGG(TAMRA)-3′
	Reverse primer: 5′-TGGCCCTTGAATCTTCTACGA-3′

[a] FAM, 5-carboxyfluorescein; TAMRA, 5-carboxytetramethylrhodamine.

2.4. Cell viability assay

Cell viability was determined at the four highest ouabain concentrations used in experiments with PHH (i.e. 3 μM, 1 μM, 0.3 μM, 0.1 μM) and the highest ouabain concentration used in experiments with stable HUH-7 hepatoma cells (i.e. 3 μM) using the ATP-based CellTiter-Glo Luminescent Cell Viability Assay (Promega G7573).

2.5. Cloning of promoter-luciferase constructs

The promoter regions of human CRP (accession number AF449713) [12,13] and human AGP (Accession No. X05779) constructs were cloned from human genomic DNA using the following primer:

CRP forward primer: 5′-ggtaccGTTAAGTAGGGAACTGTAGTAA GATTGACAGA-3′; CRP reverse primer: 5′-actcgagAAGTTCAGGGGC TAGAAGTCCTAGATCTCT-3′. AGP forward primer: 5′-ggtaccTTAC TGGAAATAGACATTCAACTTGGA-3′; AGP reverse primer: 5′-ctcgag ACTGAGACCAGGAGGCACGTGGAGCCAG-3′. The PCR fragments were subcloned, digested, and finally cloned into pGL3-basic vector and verified by sequencing.

2.6. Stable transfections

Stable transfections were performed using the FuGENE 6 reagent (Roche). Cells were co-transfected either with pGL3-CRP-luc or pGL3-AGP-luc and pcDNA3.1 expressing a neomycin resistance gene. Standard medium containing 1 mg/mL G418 (Invitrogen) was added for selection of stable clones. Stable clones were isolated after three weeks. After expansion, stable clones were tested for IL-1β and IL-6 responsiveness by determining luciferase activity. One stable clone each (HUH-CRP-luc, HUH-AGP-luc) was used for further experiments.

2.7. Determination of CRP, SAA, and IL-6 protein concentrations by ELISA

PHHs were seeded in 96 well plates. After two days medium was replaced and dose–response curves of test compounds and 10 ng/ml IL-6 were added in fresh media. Concentrations of CRP

and SAA in the supernatant of PHHs were measured after 48 h by human high-sensitivity CRP ELISA (IMUCLONE hsCRP ELISA; American Diagnostica) and human Serum Amyloid A ELISA (US Biological).

CASMCs were seeded in 96 well plates in Sm-GM. After two days medium was replaced and dose–response curves of test compounds and 0.1 ng/ml IL-1β were added in fresh media. The concentrations of IL-6 in the supernatant of CASMCs were determined after 24 h with the human high-sensitivity IL-6 ELISA (Abcam).

3. Results

3.1. Stimulation of AGP- and CRP-promoters by IL-1β and IL-6

We sought after small molecules which interfere with the IL-1β- and IL-6-induced APP expression from human hepatocytes. We used HUH-7 cells, stably transfected with fusions of either the human CRP or the human AGP-promoter and a luciferase reporter gene. Fig. 1 shows that both cytokines potently activate the respective APP promoter-luciferase construct. In fact, IL-1β was found to have a higher potency and efficacy compared to IL-6 on CRP (EC_{50}s: IL-1β 0.14 ng/ml, IL-6 4.98 ng/ml) and AGP expression, respectively (IL-1β 0.48 ng/ml, IL-6 1.45 ng/ml). We used the stable HUH-7 CRP clone and screened a library of 1280 pharmacologically active compounds (LOPAC^{1280}) for inhibitors of the IL-1β- and IL-6-induced CRP-promoter-luciferase expression. We were able to identify ouabain, an endogenous inhibitor of the Na^+/K^+-ATPase as a potent inhibitor of the IL-1β- and IL-6-induced CRP expression.

3.2. Effect of ouabain on APP promoter activity and CRP synthesis

Fig. 2 shows that ouabain potently blocks both the IL-1β- as well as the IL-6-induced CRP-promoter activity with an about 2-fold higher potency with respect to IL-1β-induced CRP expression (Fig. 2A). We found similar potencies also on the IL-1β- and IL-6-induced AGP-promoter activity, again with about 2-fold higher potency with respect to IL-1β induced AGP expression (Fig. 2B). We investigated in parallel cell viability and found no sign for toxicity at the highest concentration used for CRP- or AGP-promoter activity analysis: cell viability was 101.8 ± 2.8, 93.7 ± 2.5, 127.4 ± 10.7, and 108.8 ± 4.5 percent at 3 μM in the IL-1β induced CRP, IL-6 induced CRP, IL-1β-induced AGP and IL-6-induced AGP expression assay, respectively.

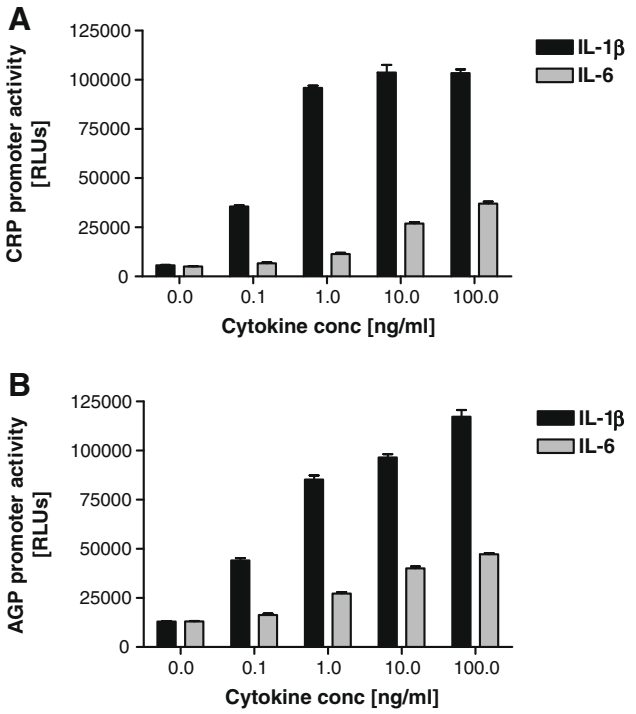

Fig. 1. Stimulation of CRP- (A) and AGP-promoters (B) by IL-1β (black bars) and IL-6 (gray bars) in stable HUH-7 hepatoma cells. Luciferase activity expressed in relative light units (RLUs) was determined after 24 h and expressed in percent values. Values from $n = 6$ determinations are given as means ± SD.

Ouabain belongs to the chemical class of cardenolide glycosides which are well known for their potent inhibitory activity of the Na^+/K^+-ATPase. We therefore analyzed the expression of the four alpha subunits of the Na^+/K^+-ATPase by using RT-PCR (Fig. 2C and D). Fig. 2C shows that the human HUH-7 CRP clone and PHH strongly express the α1 (ATP1A1) subunit, while the α2 (ATP1A2), α3 (ATP1A3), and α4 (ATP1A4) subunits are only expressed at much lower levels, if at all. ATP1A2 seems to be expressed at low levels in both HUH-7 cells and PHH while ATP1A3 and ATP1A4 are only detectable at very low levels in PHH. We then compared hepatic expression of the α1 subunit with other human tissues. Fig. 2D shows that expression of ATP1A1 in total liver, HUH-7 cells, and PHH is very similar and higher in comparison to tissues like aorta, small intestine, spleen, and skeletal muscle. Highest expression of ATP1A1 was found in brain and kidney. In summary, HUH-7 hepatoma cells and PHH strongly express the α1 subunit and to minor extents the α2 subunit of the Na^+/K^+-ATPase. Therefore, hepatocellular activity of oubain is supported by strong expression of its molecular target, the α subunit. We tested ouabain also for its ability to block simultaneously the expression of IL-6 induced CRP and SAA protein release from primary hepatocytes (Fig. 2E). Ouabain exhibits IC_{50} values for both IL-6 induced APPs that are very similar to the respective values of IL-6-induced CRP- or AGP- promoter stimulation in the stable hepatoma cells. We observed also a significant decrease in cell viability with ouabain on primary hepatocytes starting at 0.3 μM (hatched bars, Fig. 2E), a concentration at which the respective APP expression was already maximally repressed.

3.3. Inhibition of IL-1β-induced CRP-promoter stimulation by different cardiac glycosides

The inhibitory potential of cardenolide glycosides strictly depends on two major moieties at the central steroid scaffold: a glycosidic part and a C17 lactone part. Therefore, we analysed several structurally different cardenolides with or without glycosidic and C17 lactone moieties. Fig. 3 shows that cymarin, ouabain, and digitoxin, potent Na^+/K^+-ATPase inhibitors which contain a cardenolide with a C17 lactone as well as a glycosidic part, potently inhibit the IL-1β-induced CRP expression. In contrast, gitoxigenin, a cardenolide without a glycosidic part, and digitonin, a glycosidic cardenolide without a C17 lactone are almost inactive up to 3 μM on the IL-1β -induced CRP expression.

3.4. Calcium-dependence and cell type restriction of APP expression inhibition

Since cardiac glycosides increase intracellular calcium by blocking Na^+/K^+-ATPase, we wondered whether a calcium ionophore like calcimycin would also act as an inhibitor of IL-1β and IL-6 induced APP expression. Fig. 4A and B shows that calcimycin blocks the IL-1β and IL-6 induced CRP- and AGP-promoters in the human HUH-7 hepatoma cell line. Cell viability was 59.1 ± 10.3, 58.6 ± 8.7, 135.3 ± 17.5 and 55.4 ± 13.3 percent at 10 μM in the IL-1β-induced CRP, IL-6-induced CRP, IL-1β-induced AGP, and IL-6-induced AGP expression assay, respectively. We found even higher inhibitory

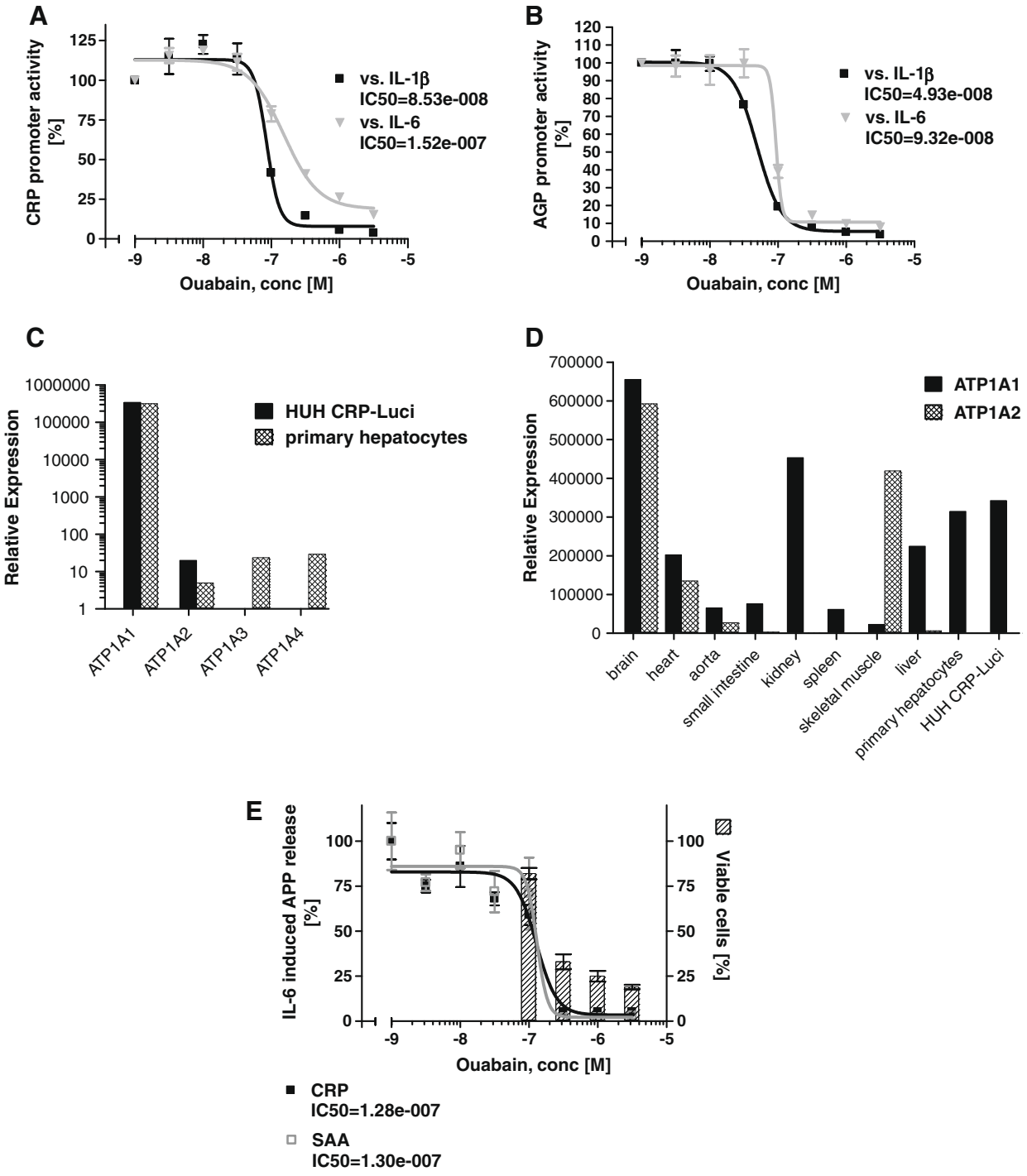

Fig. 2. Inhibition of IL-1β (black squares) or IL-6 (gray triangles) induced CRP- and AGP- promoter stimulation by ouabain in human hepatoma cells. Stable HUH-7 cells were treated in (A, CRP-promoter) and (B, AGP-promoter) with dose–response curves of ouabain in combination with 1 ng/ml IL-1β or 10 ng/ml IL-6. Luciferase activity was determined 24 h later. Values from $n = 8$ determinations are given as means ± SD. (C) Expression of the four Na^+/K^+-ATPase alpha subunits in HUH-7 CRP clone and PHH as revealed by RT-PCR. (D) Comparison of expression of the α1 and α2 subunits in human HUH-7 and PHH cells and other human tissues. (E) Inhibition of IL-6-induced CRP and SAA release by ouabain from PHHs. CRP (black squares) and SAA (gray squares) protein concentrations in the supernatant and cell viability (hatched bars, determined at the four highest compound concentrations) of PHHs were determined after stimulation with 10 ng/ml IL-6. All determined values ($n = 3$ for each single assay and concentration) were normalized and expressed in percent values. Values are given as means ± SD.

activity of calcimycin on the IL-6 induced CRP protein release from PHH (IC_{50} = 373 nM, Fig. 4C) when compared to the IL-6-induced CRP expression in HUH-7 cells (IC_{50} = 960 nM). Cell viability (hatched bars) was still about 70% at 3 μM when CRP release was inhibited already to 100%. These results support the hypothesis that hepatocellular APP release is triggered by intracellular calcium concentrations.

Since IL-1β is a potent stimulus for the release of different inflammatory proteins, e.g. from hepatocytes [13] and smooth muscle cells [14], we investigated whether the identified APP expression inhibitors may influence also the expression of cognate inflammatory proteins (i.e. IL-6) in a different cellular background. Fig. 4D shows that the IL-1β-induced IL-6 release from CASMCs is potently blocked by the anti-inflammatory control dexamethasone

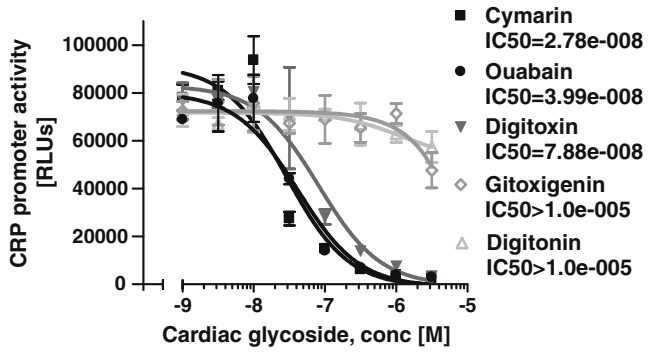

Fig. 3. Inhibition of IL-1β (1 ng/ml)-induced CRP-promoter stimulation by different cardiac glycosides. Stable HUH-7 cells were treated with dose–response curves of cardiac glycosides in combination with 1 ng/ml IL-1β. Luciferase activity expressed in relative light units (RLUs) was determined 24 h later and expressed in percent values. Values from $n = 4$ determinations are given as means ± SD.

(IC_{50}: 1.98 nM). Ouabain and calcimycin however, do not exhibit significant activity up to 1 μM here. Thus, we could confine the inhibitory effect of the cardiac glycoside ouabain to the IL-1β- and IL-6-induced APP expression from hepatocytes.

4. Discussion

In this report, we describe the known Na^+/K^+-ATPase inhibitors ouabain, cymarin and digitoxin as potent transcriptional inhibitors of hepatic acute-phase response and CRP synthesis. The demonstrated inhibitory effects on the APP transcription as deduced from reporter gene assays lead to a reduced protein secretion as determined in the supernatant from PHH. Experiments with truncated cardiac glycosides provide evidence that the inhibitory effects on APP expression strictly correlate with blockade of the Na^+/K^+-ATPase, since cardiac glycosides without a C17 lactone or without a glycosidic part are only weak Na^+/K^+-ATPase inhibitors [15]. Expression studies in human hepatoma cells and primary hepatocytes revealed strong expression of the α1 subunit of the Na^+/K^+-ATPase, which is the known molecular target for cardiac glycosides [16]. Since blocking of the Na^+/K^+-ATPase modulates cellular calcium homeostasis, we wondered whether a cellular calcium increase *per se* may interfere with the IL-1β- and IL-6-induced promoter activity of CRP and AGP. Accordingly, we predicted that a calcium ionophore like calcimycin should have a similar inhibitory effect which is indeed the case. Finally, qualified specificity of ouabain for interference with signal cascades of APP expression in hepatocytes is demonstrated since ouabain was found to be without any influence on the release of the cognate inflammatory protein IL-6 by IL-1β stimulation from primary human CASMCs.

The therapeutic effect of digitalis on dropsy was first described by William Withering in the 18th century [17] and, since then, digitalis is in medical use for the treatment of cardiac insufficiency. A long lasting search for endogenous cardiac glycosides resulted in the isolation of ouabain from human plasma [18] and the characterization of its pathophysiological role in congestive heart failure, hypertension and cancer [19]. However, although a large body of

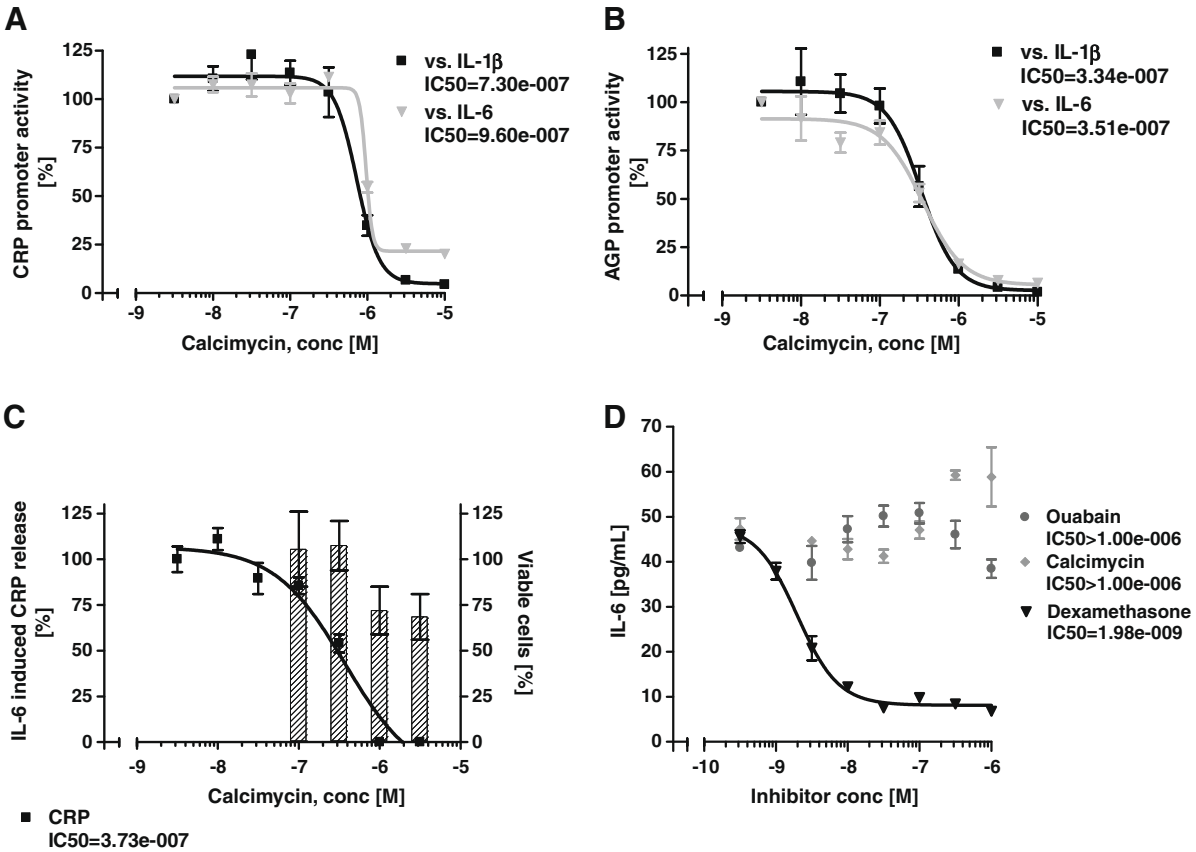

Fig. 4. Inhibition of IL-1β- or IL-6-induced CRP- and AGP-promoter stimulation by calcimycin in human hepatoma cells (A,B) and inhibition of IL-6 induced CRP release from PHH (C). Stable HuH-7 cells were treated in (A, CRP-promoter) and (B, AGP-promoter) with dose–response curves of calcimycin in combination with 1 ng/ml IL-1β or 10 ng/ml IL-6. Luciferase activity was determined 24 h later. Values from $n = 8$ determinations are given as means ± SD. CRP protein concentrations (black squares) in the supernatant and cell viability (hatched bars, determined at the four highest compound concentrations) of PHHs were determined after stimulation with 10 ng/ml IL-6 (C). All determined values ($n = 3$ for each single assay and concentration) were normalized and expressed in percent values. Values are given as means ± SD. (D) Inhibition of IL-1β-induced IL-6 release from CASMCs. IL-6 protein concentrations in the supernatant of primary human CASMCs were determined after stimulation with 0.1 ng/ml IL-1β. Values from $n = 2$ determinations are given as means ± SD.

excellent information is available on cardiac glycosides in the literature [19], an inhibitory effect of cardiac glycosides on CRP and APR has never been described before. It may be prudent to speculate that ouabain is an endogenous regulator of acute-phase response. The latter suggestion may indeed be supported by one publication showing that endogenous ouabain levels are elevated in critically ill patients with increased CRP levels [20]. Interestingly, we observed that the IC_{50} value of digitoxin in our experiments (79 nM) is only about 3-fold higher in comparison to the concentrations of 20–33 nM detected in plasma of patients with cardiac disease receiving therapeutic dosages of this drug [21]. Higher local hepatocellular concentrations of digitoxin after the first liver passage are very probable.

The therapeutic index of digoxin is narrow. We found no cytotoxicity mediated by cardiac glycosides to our stable hepatoma cells at the conditions used for investigation of IL-1β- and IL-6-induced CRP or AGP expression. Similar results have been observed by Kau et al. [22] who found that digoxin and ouabain exhibited no cytotoxicity up to 1 μM. Unfortunately, we observed the most significant decrease of cell viability with ouabain on primary hepatocytes from available donors although, even in these cells, independency of CRP and SAA inhibition from toxicity remains obvious.

CRP seems to be intimately involved in the pathogenesis of autoimmune disease [23] and atherosclerosis [3,7]. CRP shares several essential functions with antibodies (i.e. complement activation via the classical pathway [24], binding to Fcγ-receptors [1,4,5] and opsonization of biological particles for macrophages [25]) and may thus be the most primitive antibody in the evolution of the mammalian immune system. Many of the immunological functions of CRP in the immune system, during evolution, seem to have been taken over by specific antibodies and CRP may thus be considered as an atavism of the immune system. Consequently, targeting CRP represents an attractive approach [3,26] and making use of the right animal model for further *in vivo* investigations of the identified inhibitors will be the next crucial step [27]. In fact, the fat-fed and Watanabe rabbit atherosclerosis models are suitable animal models to test CRP inhibitors *in vivo* [3,28], whereas the mouse model, due to the fact that CRP is not an acute-phase reactant in mice, is obviously not as helpful [29]. In 2005, Jianglin Fan and colleagues reported substantial correlation between plasma CRP and the degree of atherosclerosis in cholesterol-fed rabbits as well as deposition of the rabbit CRP in atherosclerotic lesions of these animals [3,28]. Recent research with transgenic (Tg) rabbits expressing human CRP (hCRP) revealed that the additional plasma hCRP did not affect aortic or coronary atherosclerosis lesion formation [30]. The marked increase in rabbit CRP induced by hypercholesterolemia [28], however, may already have maximum effects.

The inhibitory activity of cardiac glycosides is not CRP specific and also visible for other APPs like AGP and SAA. Pleiotropic actions of cardiac glycosides even independent of Na^+/K^+-ATPase inhibition have been observed very recently. Notably, digitoxin potently blocks the interaction of TNF receptor-associated death domain with TNF receptor 1 resulting in an inhibition of the TNF-alpha/NF-κB signaling pathway [31]. Our novel observations therefore build on the concept that further optimization of this fascinating chemical class of compounds may ultimately lead to more specific anti-inflammatory drugs and/or regulators of acute-phase response.

In summary, our report may open up new horizons in CRP research because it describes the first transcriptional CRP inhibitors acting at the nanomolar range.

Funding

This work was supported by BAYER HealthCare, Wuppertal, Germany.

Disclosures

None.

Acknowledgments

We thank Dr. Stefan Golz and Dr. Frank Kramer for discussions. We gratefully acknowledge Dr. David Bowyer, Cambridge, UK for critical reading and substantial improvement of the paper.

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