JCI The Journal of Clinical Investigation

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J Clin Invest. 2025. https://doi.org/10.1172/JCI186937.

Research Letter In-Press Preview Cell biology Pulmonology

Serum cAMP levels are increased in patients with asthma

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As a cornerstone in the treatment of asthma, β_2-agonists prevent or reverse the shortening of human airway smooth muscle (HASM), the pivotal cell regulating bronchomotor tone. β_2-agonists act upon β_2-adrenoceptor (β_2AR)–the cognate G_s-coupled G protein-coupled receptor (G_s-GPCR) expressed on HASM–and activate adenylyl cyclase which generates 3',5'-cyclic adenosine monophosphate (cAMP) (1). Increased intracellular cAMP levels ($[cAMP]_i$) consequently stimulate protein kinase A that in turn modulates multiple downstream targets to promote HASM relaxation and reverse airflow obstruction (2).

Classically, the signal transduction evoked by β_2ARs is short-lived and multiple mechanisms ensure homeostatic regulation of $[cAMP]_i$, with phosphodiesterase (PDE) degradation of cAMP considered to play a dominant role. Using primary HASM cells in culture as a model, we recently reported that β_2AR activation evokes cAMP egress to the extracellular space ($[cAMP]_e$) that is long-lived in culture, independent of PDE activity, and mediated by ABCC1 (ATP-binding cassette subfamily C member 1) membrane transporter (3). Inhibition of ABCC1 activity or expression decreases cAMP egress, increases $[cAMP]_i$ and enhances HASM relaxation elicited by structurally diverse agonists acting upon G_s-GPCRs (3). These findings suggest a class effect of G_s-GPCR activation and identify ABCC1 as previously unrecognized cAMP signal response modifier in HASM. Of note, in a small cohort of patients with and without asthma, we detected increased cAMP levels in the blood of patients with asthma (3).

To further explore the clinical utility of detecting circulating cAMP, we measured cAMP levels in a serum biobank from the Severe Asthma Research Program (SARP)-3 (4). For this study, we obtained 87 serum samples of patients with asthma, of which 39 are characterized as "severe" according to European Respiratory Society / American Thoracic Society criteria for asthma severity (Supplementary Table 1–2) (5). Since SARP-3 did not have a sufficient serum biobank of healthy controls, we leveraged the database from the Rutgers Corona Cohort (RCC) study (6) and obtained 273 serum samples of the study participants without a known history of

asthma or other lung diseases shown in Supplementary Table 1 (see detailed inclusion and exclusion criteria in Supplementary Information).

We detected a high variability or a wide spread of cAMP levels in 87 serum samples of patients with asthma (Figure 1A), ranging from 0.291 to 563.9 picomole. In contrast, the range of cAMP levels in 273 serum samples of individuals without asthma was markedly smaller (0 to 27.72 picomole) (Figure 1A). Compared with non-asthma group (0.520 picomole, median), serum cAMP levels were significantly higher in patients with asthma (6.220 picomole, median) (Supplementary Table 1). To further test the hypothesis that cAMP levels can differentiate asthma severity (severe versus nonsevere) in the SARP-3 samples, we applied linear regression models with age and sex as covariates across clinical groups. There was no significant difference of serum cAMP levels between severe asthma and nonsevere asthma (Supplementary Table 2), but each asthma group showed significantly higher cAMP levels (adjusted $P < 0.00001$) than non-asthma group (Figure 1B).

Using the SARP-3 data, we next assessed whether measured serum cAMP levels are associated with any clinical traits of asthma. Specifically, we asked whether serum cAMP levels had associations with: 1) asthma endotypes; 2) poor control indicators; and 3) post-bronchodilator airflow reversibility. There were no significant differences in cAMP levels among or between groups stratified by eosinophilic or neutrophilic asthma (Supplementary Figure 1) and any of the poor control indicators (Supplementary Figure 2). In addition, we did not detect significant differences in serum cAMP levels with maximum FEV_1 reversibility with albuterol (Supplementary Figure 3A). Of note, serum cAMP levels arithmetically increased with the number of inhaled corticosteroids (ICS) puffs and controllers used (Supplementary Figure 1) and, in nonsevere asthma group, increased with the increases of post-bronchodilator lung function (Supplementary Figure 3B-C). Further studies are necessary to explore the link between serum cAMP levels with: 1) bronchodilator or treatment responses by asthma severity; 2) ABCC1 expression and activity in health and disease, including specific cell types of origin; and, 3) whether these physiological

outcomes and clinical phenotypes are affected by variations in ABCC1 genotypes in a large cohort of patients with and without asthma.

Acknowledgement: Supported by UL1TR0030117, P01HL114471 and R01HL164404. The authors would like to thank RCC and SARP participants and investigators, including the clinical coordinators and laboratory personnel for their contribution to the study.

Disclosure: The following companies provided financial support for study activities at the Coordinating and Clinical Centers beyond the third year of patient follow-up: AstraZeneca, Boehringer-Ingelheim, Genentech, GlaxoSmithKline, Sanofi–Genzyme–Regeneron, and TEVA. These companies had no role in study design or data analysis, and the only restriction on the funds was that they be used to support the SARP initiative.

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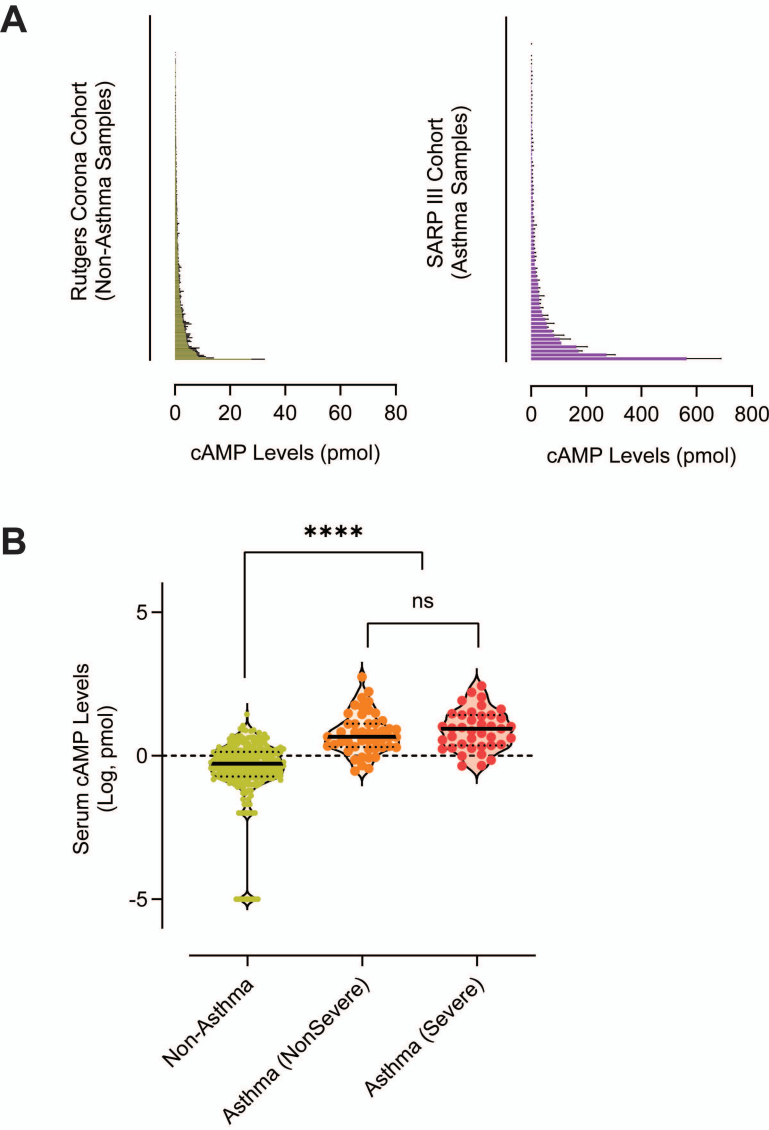
FIGURE LEGENDS

Figure 1: Serum cAMP levels are increased in patients with asthma. (**A**) Histogram of cAMP levels detected in the blood of RCC study participants without a known history of asthma or other lung diseases (N=273) and patients with asthma in the SARP-3 (N=87). For each sample, cAMP levels (pmol per 60 μl serum) were measured in duplicates by using cAMP-Screen System ELISA kit (Applied Biosystem) and presented as Mean ± SD. (**B**) Serum cAMP levels by asthma severity. Asthma severity was defined as "nonsevere" (N=48) and "severe" (N=39) according to ERS/ATS criteria. Linear regression models were used with age and sex as covariates across clinical groups. To satisfy the normalization assumption necessary for linear regression testing, cAMP levels underwent log transformation and the analysis was conducted using R version 4.4.1.


A
Rutgers Corona Cohort
(Non-Asthma Samples)
SARP III Cohort
(Asthma Samples)
0
20
40
60
80
cAMP Levels (pmol)
0
200
400
600
800
cAMP Levels (pmol)
B
****
ns
Serum cAMP Levels
(Log, pmol)
5
0
-5
Non-Asthma
Asthma (NonSevere)
Asthma (Severe)