ELSEVIER

European Journal of Pharmacology 384 (1999) 239–241

ejp

www.elsevier.nl/locate/ejphar

Short communication

The influence of isatin on guanylyl cyclase of rat heart membranes

Alexei Medvedev [a,*], Merton Sandler [b], Vivette Glover [c]

[a] *Institute of Biomedical Chemistry, Academy of Medical Sciences, 10 Pogodinskaya Str., 119832 Moscow, Russia*
[b] *Department of Chemical Pathology, Queen Charlotte's and Chelsea Hospital, London, UK*
[c] *Department of Maternal–Fetal Medicine, Queen Charlotte's and Chelsea Hospital, London, UK*

Received 24 September 1999; accepted 28 September 1999

Abstract

The influence of indole-2,3 dione (isatin) on particulate guanylyl cyclase (GC) from rat heart membranes was investigated in the presence of adenylylimidodiphosphate (AMP-PNP). The latter activated GC in a concentration-dependent manner and 100 μM isatin abolished this effect. The IC_{50} value, 2 μM, for the inhibition of stimulation of GC induced by 50 μM AMP-PNP, was close to the upper physiological level of isatin. These results indicate that isatin may interact with GC independently of its regulation by natriuretic peptides.

Keywords: Isatin; Natriuretic peptide receptor; Guanylyl cyclase, Particulate; Adenylylimidodiphosphate (AMP-PNP)

1. Introduction

Isatin (indole-2,3 dione) has a wide range of pharmacological actions (Medvedev et al., 1996; Glover et al., 1998). It is present in the brain, heart and other tissues at a concentration (up to 1–10 μg/per gram wet weight) likely to have physiological effects (Watkins et al., 1990; Medvedev et al., 1996). We have previously demonstrated that isatin inhibits atrial natriuretic peptide (ANP) receptor binding and ANP-stimulated guanylyl cyclase (GC) (Glover et al., 1995; Medvedev et al., 1996, 1998), the IC_{50} value for the former being 0.4 μM, well within the physiological range.

The natriuretic peptides are a structurally related family containing a 17-amino acid ring, stabilised by a disulphide bond (Anand-Srivastava and Trachte, 1993; Yandle, 1994). They all (ANP, brain natriuretic peptide [BNP], C-type natriuretic peptide [CNP], urodilatin) have amino terminal and carboxy terminal extensions of different lengths with the exception of CNP which lacks the carboxy terminal extension (Anand-Srivastava and Trachte, 1993; Yandle, 1994). Several types of natriuretic peptide receptors have been identified. Natriuretic peptide receptor type A and B, single transmembrane proteins of 120–130 kDa, includes membrane-bound GC, whereas C-type receptor (the clearance receptor) is coupled to adenylyl cyclase inhibition through the G_i regulatory protein (Anand-Srivastava and Trachte, 1993; Leitman et al., 1994; Savoie et al., 1995). Both ANP and BNP act at A-type of receptor (ANP_A receptor), whereas CNP interacts relatively selectively with natriuretic peptide receptor type B (Yandle, 1994). These three peptides may interact at C-type receptor and inhibit adenylyl cyclase activity in the presence of guanine nucleotide, GTP or its non-hydrolysable analogue (Anand-Srivastava, 1997; Anand-Srivastava and Trachte, 1993; Savoie et al., 1995).

Since isatin abolishes GC stimulation by ANP and BNP, we have concluded that it can interact with ANP_A receptor (Medvedev et al., 1998). It is now well established that activation of ANP_A receptor (GC A) by ANP is potentiated by ATP or its non-hydrolysable analogues such as adenylylimidodiphosphate (AMP-PNP) (Leitman et al., 1994; Potter and Hunter, 1998). The latter binds to a putative site within the cytoplasmic region of ANP_A receptor that has a homology with tyrosine kinase, but lacks tyrosine kinase activity (Leitman et al., 1994). The allosteric interaction of ATP with the intracellular kinase-like domain may activate the catalytic domain (Leitman et al., 1994; Wong et al., 1995; Potter and Hunter, 1998). We have shown previously that AMP-PNP potentiates the effect of submaximal doses of ANP and BNP and attenuates or abolishes sensitivity to isatin (Medvedev et al.,

* Corresponding author. Tel.: +7-095-246-1641; fax: +7-095-245-0857.
E-mail address: medvedev@ibmh.msk.su (A. Medvedev)

0014-2999/99/$ - see front matter
PII: S0014-2999(99)00688-3

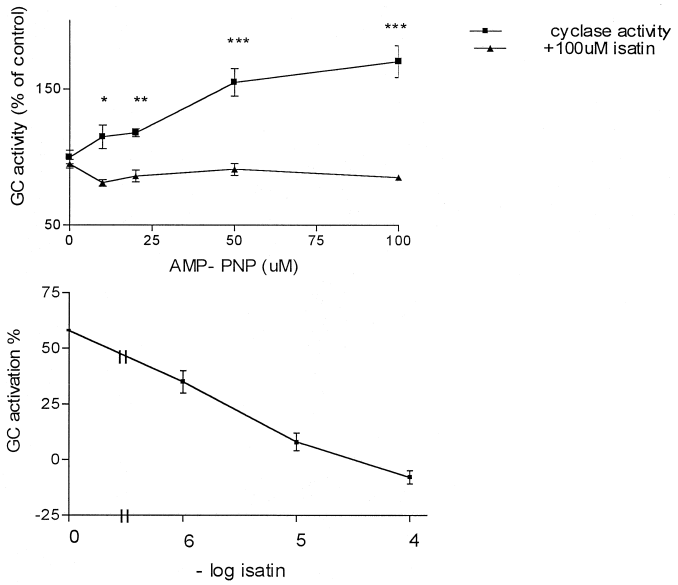

Fig. 1. (a) The influence of AMP-PNP and isatin on GC activities of rat heart membranes. Data expressed as percentage of basal cyclase activity represent mean ± S.E.M. of 3–10 experiments. Asterisks show statistical significance of isatin inhibition: $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$. The basal GC activity was 19.5 ± 1.8 pmol/10 min/mg of protein. (b) The influence of isatin on GC activation induced by 50 μM AMP-PNP. Stimulation of GC by 50 μM AMP-PNP (+55%) was defined as 100%. Data represent mean ± S.E.M. of four experiments.

1998). This finding suggests that the sensitivity of ANP_A receptor (GC A) to isatin can be allosterically regulated (perhaps at the tyrosine kinase-like domain). If the effect of isatin on ANP_A receptor were mediated allosterically in this way, we might expect that, in the absence of ANP, GC activation by ATP (or its non-hydrolysable analogue) would be resistant to isatin action. Thus, in the present study, we have investigated the influence of isatin on GC from rat heart membranes in the presence of AMP-PNP.

2. Materials and methods

Particulate GC activity was determined in rat heart membranes obtained by centrifugation at 100,000 × *g* (4°C, 1 h) of homogenates (10%, w/v) prepared in 50 mM tris–HCl buffer, pH 7.6, containing 150 mM NaCl, 1 mM EDTA, and 0.01% bacitracin. Pellets were resuspended in 50 mM tris–buffer (pH 7.6) containing 0.01% bacitracin and centrifuged as described above. The final pellets were resuspended in 50 mM tris–HCl buffer (pH 7.6) and 10–15 μg of protein was used for determination of GC activity (Medvedev et al., 1998). The reaction mixture (final volume 200 μl) contained 50 mM tris–HCl buffer, pH 7.6, 4 mM $MgCl_2$, 1 mM GTP, 1.0 mM isobutylmethylxanthine, 0.01% bacitracin and a GTP-regenerating system consisting of 15 mM creatine phosphate and 40 μg creatine kinase. Tubes were incubated at 37°C for 10 min and the reaction was stopped by adding 1.8 ml cold 50 mM sodium acetate, pH 4.8. Samples were boiled for 3 min and then centrifuged at 2000 × *g* for 30 min. The amount of cGMP generated was determined by radioimmunoassay. Under these experimental conditions, accumulation of either cGMP was linear over the first 10 min of incubation at 37°C. Isatin itself had no effect on basal GC activity up to 10^{-4} M concentration.

Radioimmunoassay kits for cGMP were obtained from the Radiochemical Centre (Amersham, UK). Other chemicals were purchased from Sigma (Poole, UK).

Statistical differences were evaluated using the Student's *t*-test.

3. Results

Fig. 1a shows that significant stimulation of particulate GC was observed at 50 μM AMP-PNP and an increase in AMP-PNP concentration to 100 μM resulted in only a small (and statistically insignificant) further increase of GC activity. This is consistent with previous observations from other laboratories (Anand-Srivastava and Trachte, 1993). 100 μM isatin inhibited this activation to slightly below the basal level. Fig. 1b shows a dose–response curve of the isatin effect on GC activated by 50 μM AMP-PNP. The IC_{50} of the AMP-PNP-stimulated GC inhibition by isatin was 2 μM, which is close to upper physiological level (Watkins et al., 1990).

4. Discussion

The present findings show that in the absence of ANP, isatin can still inhibit the activity of membrane-bound GC A stimulated by AMP-PNP. We have previously demonstrated that isatin inhibits ANP-stimulated GC and that low concentrations of AMP-PNP decrease sensitivity to isatin (Medvedev et al., 1998). This pointed to the involvement of allosteric GC regulation by isatin at a tyrosine kinase domain. If isatin were to interact only with such allosteric

sites, we would expect AMP-PNP-dependent activation of GC to be resistant to inhibition by isatin. However, isatin inhibited AMP-PNP-stimulated GC with similar efficacy to ANP-stimulated GC (Glover et al., 1995, Medvedev et al., 1998). The result can be explained if isatin were to interact with ANP_A receptor *at two sites*, both the natriuretic peptide binding site and the tyrosine kinase-like domain, the IC_{50} of the former being 0.4 μM and the latter 2 μM. The simultaneous presence of saturating concentrations of ligands that bind at these sites fully protects ANP_A receptor against isatin, as we have observed earlier (Medvedev et al., 1998). If one of these sites were vacant, isatin might exert its effect by inhibiting ANP-receptor binding or ATP (AMP-PNP)-stimulated GC activity. Other examples of one ligand, such as cGMP, binding at two distinct sites (catalytic and allosteric), are known in the cyclic nucleotide field, as with cGMP-specific phosphodiesterase (Corbin et al., 1998).

Acknowledgements

This study was partially supported by the Royal Society, INTAS (grant No. 97-1818), and the Russian Foundation for Basic Research (grant No. 97-0-48320).

References

Anand-Srivastava, M.B., 1997. Atrial natriuretic peptide-C receptor and membrane signalling in hypertension. J. Hypertens. 15, 815–826.

Anand-Srivastava, M.B., Trachte, G.J., 1993. Atrial natriuretic factor receptors and signal transduction mechanisms. Pharmacol. Rev. 45, 455–497.

Corbin, J.D., Beasley, A., Turko, I.V., Haik, T.L., Mangum, K.A., Wells, J.N., Francis, S.H., Sekhar, K.R., 1998. A photoaffinity probe covalently modifies the catalytic site of the cGMP-binding cGMP-specific phosphodiesterase. Cell Biochem. Biophys. 29, 145–157.

Glover, V., Medvedev, A., Sandler, M., 1995. Isatin is a potent endogenous antagonist of guanylate cyclase coupled atrial natriuretic peptide receptors. Life Sci. 57, 2073–2079.

Glover, V., Bhattacharya, S.K., Charkrabarti, A., Sandler, M., 1998. The psychopharmacology of isatin: a brief review. Stress Med. 14, 225–229.

Leitman, D.L., Waldman, S.A., Murad, F., 1994. Regulation of particulate guanylate cyclase by natriuretic peptides and Escherichia coli heat-stable enterotoxin. Adv. Parmacol. 26, 67–86.

Medvedev, A.E., Clow, A., Sandler, M., Glover, V., 1996. Isatin: a link between natriuretic peptides and monoamines? Biochem. Pharmacol. 52, 385–391.

Medvedev, A.E., Sandler, M., Glover, V., 1998. Interaction of isatin with type-A of natriuretic peptide receptor. Life Sci. 62, 2391–2398.

Potter, L.R., Hunter, T., 1998. Phosphorylation of kinase homology domain is essential for activation of the A-type natriuretic peptide receptor. Mol. Cell. Biochem. 18, 2164.

Savoie, P., de Champlain, J., Anand-Srivastava, M.B., 1995. C-type natriuretic peptide and brain natriuretic peptide inhibit adenylyl cyclase activity: interaction with ANF-R2/ANP-C receptors. FEBS Lett. 370, 6–10.

Watkins, P., Clow, A., Glover, V., Halket, J., Przyborowska, A., Sandler, M., 1990. Isatin, regional distribution in rat brain and tissues. Neurochem. Int. 17, 321–323.

Wong, S.K., Ma, C.P., Foster, D.C., Chen, A.Y., Garbers, D.L., 1995. The guanylyl cyclase-A receptor transduces an atrial natriuretic peptide/ATP activation signal in the absence of other proteins. J. Biol. Chem. 270, 30818–30822.

Yandle, T.G., 1994. Biochemistry of natriuretic peptides. J. Intern. Med. 235, 561–576.