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SHORT COMMUNICATION

Efficacy of Isatin Analogues as Antagonists of Rat Brain and Heart Atrial Natriuretic Peptide Receptors Coupled to Particulate Guanylyl Cyclase

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ABSTRACT. Isatin is an endogenous indole and an inhibitor of atrial natriuretic peptide (ANP) receptors coupled with particulate guanylyl cyclase (GC). In this study, several isatin analogues were tested as inhibitors of ANP-stimulated GC in rat brain and heart membranes. None of these analogues affected activity in the absence of ANP, or stimulated ANP-induced activity. In both tissues, some 5-substituted isatins (5-hydroxyisatin, 5-methylisatin, and 5-aminoisatin) exhibited more effective inhibitory activity than isatin itself, with IC_{50} values in the range 1.3–20 μM. The efficacy of other analogues varied and was not consistent between the two tissues, raising the possibility of receptor heterogeneity and relative selectivity of inhibition. Some substituted isatins may have a role as pharmacological tools for investigating the physiological roles of natriuretic peptides and their receptors. BIOCHEM PHARMACOL **57**;8:913–915, 1999. © 1999 Elsevier Science Inc.

KEY WORDS. isatin; isatin analogues; atrial natriuretic peptide; guanylyl cyclase

Isatin is an endogenous compound, widely distributed in mammalian tissues and body fluids. It has a distinct and discontinuous distribution in rat brain (see [1] for review), and its concentration in the hippocampus is approximately 0.1 μg/g, or about 1 μM [1, 2]. The substance was discovered as a component of the endogenous MAO[‖] inhibitory activity, tribulin, and is a selective inhibitor of MAO B, with an apparent inhibition constant of 3–20 μM; inhibition of MAO A occurs at higher concentrations (K_i 60–70 μM) [1]. However, *in vivo* administration of a low dose of isatin (10 mg/kg) failed to protect either form of brain MAO against irreversible inhibition by phenelzine [3]. A high dose (80 mg/kg) reduced phenelzine-dependent inhibition of MAO B but not of MAO A [3].

The most potent known action of isatin *in vitro* is its strong inhibition of ANP binding to its receptor [4], with an IC_{50} value of 0.4 μM, within the physiological range of isatin concentration in the brain [4]. Isatin has been shown to inhibit ANP-stimulated GC of rat brain, heart, and kidney membrane in a dose-dependent manner, reducing formation of cGMP [4]. It is the only known endogenously generated non-peptide compound of mammalian origin which acts as an antagonist of the natriuretic peptide system. The polysaccharide HS-142-1 [5], which competitively and selectively inhibits ANP binding to its GC-containing receptor and has been used in experimental pharmacology, is of microbial origin [6].

There is increasing evidence that isatin can exert a functional antagonism of ANP *in vivo* [7, 8]. For example, it can counteract the anxiolytic effect of ANP at a dose of 20 mg/kg [7]. Screening of isatin derivatives and analogues might reveal compounds with higher efficacy than that of isatin. In the present study, we investigated the inhibitory efficacy of a range of isatin analogues on ANP-stimulated particulate GC from rat brain and heart.

MATERIALS AND METHODS

Particulate GC activity was determined in rat brain and heart membranes by centrifugation at 100,000 *g* (4°, 1hr) of homogenates prepared in 50 mM Tris–HCl buffer pH7.6, containing 150 mM NaCl, 1 mM EDTA, and 0.01% bacitracin. Pellets were resuspended in the same buffer and washed by centrifugation. The final pellets were resuspended in 50 mM Tris–HCl buffer, and 10–15 μg of protein was used for determination of GC activity as described previously [4] with minor modifications. Briefly, the reaction mixture contained 50 mM Tris–HCl buffer, pH 7.6, 4 mM $MgCl_2$, 1 mM GTP, 1 mM isobutylmethylxanthine, 0.01% bacitracin, and a GTP-regenerating system consisting of 15 mM creatine phosphate and 40 μg creatine kinase. The amount of cGMP generated was

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‖ *Abbreviations:* ANP, atrial natriuretic peptide; GC, guanylyl cyclase; MAO, monoamine oxidase; cGMP, cyclic GMP; and NPR, natriuretic peptide receptor.

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TABLE 1. Inhibition (%) of ANP-dependent activation of particulate guanylyl cyclase from rat brain and heart by isatin analogues

Compound (50 μM)	Brain	Heart
Isatin	79.1 ± 1.9	49.3 ± 5.8
5-Aminoisatin	93.9 ± 6.1*	90.6 ± 5.9‡
5-Hydroxyisatin	75.0 ± 9.0	59.7 ± 13.4
5-Methylisatin	100 ± 0‡	74.7 ± 6.7*
5-Ethylisatin	95 ± 2.7†	49.6 ± 9.8
5-Propylisatin	39.2 ± 9.5†	37.7 ± 2.2
5,6-Dimethylisatin	1.7 ± 1.7‡	14 ± 4.1†
4,5,6-Trimethoxyisatin	62.1 ± 9.6*	50.3 ± 13.4
6-Hydroxyisatin	54.4 ± 21.0	16.7 ± 4.2*
7-Ethylisatin	5.0 ± 3.5‡	70.3 ± 8.8
N-Methylisatin	33.7 ± 17.9	24.0 ± 14.1
N-Acetylisatin	0‡	64.5 ± 8.0

Mean ± SEM from 3–5 experiments. The GC stimulation by 1 μM ANP was defined as 100% in each tissue. Actual stimulation over baseline: brain 35.7 ± 5.0%; heart 60 ± 4.7%.

*$P < 0.05$, †$P < 0.02$, and ‡$P < 0.001$ (paired Student's *t*-test, indicating significant difference between effect of isatin and its analogues.

determined by radioimmunoassay. Basal activity was stimulated by 1 μM ANP, which produced maximal stimulation of GC activity in all tissues studied [4].

The influence of isatin and some of its analogues on the binding of 40 pM ^{125}I-ANP to rat brain preparations was performed as described previously [4].

Radioimmunoassay kits for cGMP determination and ^{125}I-ANP for the binding experiments were purchased from the Radiochemical Centre, Amersham.

Isatin derivatives were prepared by standard methods [9–12] by Brian L. Goodwin, modified, where necessary, for preparation of hydroxy- and *N*-substituted isatins. All structures were confirmed by mass spectral analysis [13]. Isatin and its analogues dissolved well in water with moderate heating (50–60° for 2 min).

RESULTS AND DISCUSSION

The effects of 50 μM isatin and some of its analogues on ANP-stimulated GC activity are given in Table 1. Maximal stimulation of GC was obtained with 1 μM ANP and this was considered as 100%. The absolute values for the stimulation over baseline were 35.7 ± 5.0 and 60 ± 4.7% for brain and heart, respectively. This is consistent with previously published data [4]. At 50 μM, none of the isatin analogues inhibited or stimulated basal GC activity. Fifty μM isatin reduced the stimulation by ANP of particulate GC from rat brain and heart by 60–80%. This is also consistent with our previous data [4], which found that the total inhibition of ANP-stimulated GC activity was achieved by 100 μM isatin. It should be noted that this system, which involved maximal stimulation of GC by a high concentration of ANP (1 μM), is different from that used for the direct inhibition of ANP binding to its receptor by isatin. The latter gave an IC_{50} of 0.4 μM, and used 40 pM ^{125}I-ANP [4].

Table 1 shows that a substituent in the 5 position can increase the efficacy of inhibition in both tissues. The 5-substituted isatins, 5-aminoisatin, 5-hydroxyisatin, and 5-methylisatin (all at 50 μM), demonstrated greater inhibitory efficacy in both heart and brain ANP-stimulated GC activity than did isatin itself. The inhibitory properties of the other analogues varied and also manifested some tissue variations. *N*-acetylisatin and 7-ethylisatin were much more effective inhibitors of ANP-stimulated GC from the heart, whereas 5-ethylisatin and 6-hydroxyisatin were more effective in the brain. The correlation between the whole series on the two tissues was only 0.4 (Spearman–Rank, not significant).

For the more effective inhibitors, we determined IC_{50} values (the concentration required for 50% decrease of *in vitro* stimulation of GC by 1 μM ANP), using 4 different concentrations (1, 10, 50, and 100 μM). In the brain, they were (μM): 4.0 (for 5-aminoisatin), 5.0 (for 5-ethylisatin), 7.9 (5-methylisatin), 16 (for isatin), and 22 (for 5-hydroxyisatin). In the heart, the IC_{50} values were (μM): 1.3 (5-hydroxyisatin), 6.3 (5-methylisatin), 7.9 (5-aminoisatin), 12.6 (6-ethylisatin), 20.0 (7-ethylisatin), and 22 (isatin).

To confirm that the analogues were acting by inhibiting the effects of ANP, rather than directly on particulate GC, we compared the inhibitory effects of 10 μM 5-methylisatin, 5-ethylisatin, and isatin on the specific binding of ^{125}I-ANP to a rat brain membrane fraction. The compounds tested exhibited the same rank of inhibitory potency as with the ANP-stimulated GC from rat brain: 5-methyisatin (inhibition by 73%) >5-ethylisatin (inhibition by 53%) >isatin (inhibition by 44%) (N = 2).

Previously, we found that isatin inhibits ANP-stimulated particulate GC in both brain and heart in a concentration-dependent manner, although there were some tissue differences in inhibition pattern [4]. One explanation of the present finding of different effects of particular analogues in brain and heart would be in terms of receptor heterogeneity. Two types of NPR coupled to GC have been identified, NPR-A and NPR-B [14]. The former is activated by natriuretic peptides in the following order: ANP>BNP≫CNP, whilst NPR-B is selective for CNP, with a rank order of stimulating potency of CNP>ANP> BNP [15]. High concentrations of ANP, as used in this study, can probably exert some stimulating influence on NPR-B as well as NPR-A. Each has been recognised in both tissues, with some suggestion that the brain is relatively richer in NPR-A [14–17]. It may be that certain of the isatin analogues have preferential effects on each subtype. This hypothesis clearly needs further research, but raises the possibility that some of these analogues, or other similar derivatives, may be useful in delineating the roles of the two types of receptor.

Some 5-substituted isatins, namely 5-hydroxyisatin, 5-methylisatin, and 5-ethylisatin, also exhibited more potent inhibition of MAO A and (with the exception of 5-hydroxyisatin) MAO B than isatin [13, 18]. Substituents

at other positions of the isatin ring (6-hydroxy-, 7-hydroxy-, and *N*-methyl-) decreased inhibitory activity of the compounds with respect to both forms of MAO.

In conclusion, the present findings suggest that some substituted isatins may be useful as pharmacological tools for the investigation of the physiological role of ANP. 5-Hydroxyisatin, 5-methylisatin, and 5-aminoisatin were more potent than isatin in both tissues studied. We need to investigate whether they can inhibit ANP-dependent cGMP accumulation in intact cells. It will also be of interest to investigate whether certain of the other analogues have a selective action in particular tissues, for example *N*-acetylisatin in the heart, and 5-ethylisatin in the brain, when tested *in vivo*.

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