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Short communication

Cyclic GMP excretion blocked by isatin administration under conditions of fluid overload

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Abstract

Isatin is a potent inhibitor of atrial natriuretic peptide (ANP) receptors and ANP-induced generation of cGMP *in vitro*. This study was designed to determine whether it had a similar effect *in vivo*, using a model of fluid overload known to induce ANP. We confirmed that this model increased urinary output of cGMP 3 hr after volume loading, and showed that this effect was blocked by i.p. injection of isatin (50 mg/kg). Isatin had no effect on urine volume or sodium output. However, isatin did have an effect on plasma protein concentration, both compared with control values, compatible with shifting fluid to the vascular compartment, and after volume overload, in which it normalised such a shift. Isatin thus affected both the generation of cGMP and fluid balance *in vivo*.

Keywords: ANP; cGMP; Water balance; Isatin; Rat; Plasma

1. Introduction

Isatin is an endogenous compound widely distributed in mammalian tissues and body fluids [1–3]. It has a distinct and discontinuous distribution in rat brain, and its concentration in the hippocampus is approximately 0.1 μg/g, or about 1 μM [4]. The most potent known action of isatin, *in vitro*, is its inhibition of ANP binding to its receptor, with an IC_{50} value of 0.4 μM, within the physiological range of isatin concentration in the brain [5]. Isatin has been shown to inhibit ANP-stimulated guanylate cyclase of rat brain, heart, and kidney membranes in a dose-dependent manner, reducing formation of cGMP [5]. It is the only known endogenously generated non-peptide compound of mammalian origin that acts as an antagonist of the natriuretic peptide system. The microbial HS-142-1 [6], which competitively and selectively inhibits GC-coupled ANP binding to its receptor and has been used in experimental pharmacology, is a polysaccharide. There is some evidence that isatin can exert a functional antagonism of ANP *in vivo*. For example, it can counteract the anxiolytic effect of ANP at a dose of 20 mg/kg [7]; it can also antagonise behavioural effects of brain natriuretic peptide (BNP) and C-type natriuretic peptide (CNP) *in vivo* [8]. However, there has been no evidence, to date, as to whether it can block ANP-induced generation of cGMP *in vivo*.

ANP is known to be involved in water and electrolyte homeostasis; one of its roles appears to be to protect the body against fluid overload [9]. Studies in humans and experimental animals have shown that volume overload increases release of ANP [10]. Effects of ANP are mediated by receptors coupled to guanylate cyclase, which generates cGMP [11]. Thus, in this study, we used the physiologically relevant model of volume loading and tested the effect of isatin on cGMP output, together with plasma protein concentration and urine volume and sodium excretion.

2. Materials and methods

2.1. Animals

Adult male Wistar rats weighing approximately 240 g were used. The animals were housed in groups of 5–7 in

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Abbreviation: ANP, atrial natriuretic peptide.

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Table 1
Effects of acute volume overload and isatin after 3 hr. Mean + SE (n = 12)

	Control	Control + isatin	Overload	Overload + isatin	One-way Anova *P*
Urine cGMP pmol/total volume	1365 + 242	1889 + 326	4958 + 850***	2336 + 404##	<0.0001
Plasma cGMP pmol/g protein	191 ± 12.1	240 ± 25.4	307 ± 43.4	243 ± 37.5	0.0994
Urine Na+ μmol/total volume	86 ± 16	92 ± 19	816 ± 106***	880 ± 137	<0.0001
Urine volume mL	1.1 ± 0.2	1.26 ± 0.33	9.35 ± 2.98**	6.64 ± 1.19	0.0011
Plasma protein mg/100 mL	76.1 + 2.7	65.1 + 1.7**	58.6 + 1.6***	67.5 + 2.3#	<0.0001

After Bonferroni's multiple comparison test. ** $P < 0.01$; *** $P < 0.001$ compared with control # $P < 0.05$; ## $P < 0.01$ compared with overload.

cages in a room maintained at constant temperature (23 ± 1°) and on a 12-hr dark–light cycle (lights on from 6 a.m. to 6 p.m.) with free access to tap water and standard laboratory food. The animals were kept and handled during the experiments in accordance with the instructions of Albert Szent-Györgyi Medical University Ethical Committee for the Protection of Animals in Research.

2.2. Treatments

Sterile pyrogen-free saline (0.9%, Biogal) was injected i.p. in a volume weighing 10% of the body. Isatin (indole-2, 3-dione, Sigma Chemical Co.) was diluted in saline and administered i.p. at a dose of 50 mg/kg 30 min before the volume overload. This dose was chosen because it was effective in the behaviour study [8]. One millitre isatin solution contained 20 mg isatin (dissolved after heating and administered after cooling to body temperature). The control animals received the same volume of saline for all experiments.

2.3. Procedures

Acute volume overload was initiated by injecting saline (10% of body weight) i.p. to the animals. Rats were then separately placed into chambers and urine was collected from each animal for 3 or 5 hr, respectively. Three or five hours after acute volume overload, trunk blood samples were collected from decapitated animals into test tubes containing EDTA. One milliline of 5% EDTA solution was added to 9 mL of blood. Both blood and urine samples were centrifuged (3000 *g* for 10 min) and supernatants stored at −20° until further use. Urine sodium concentration was determined from each sample using flame photometry. Serum and urine cGMP concentration were determined by standard radioimmunoassay (cGMP ^{125}I assay kits, Amersham). Protein concentration in plasma was determined by the Lowry method. The following experiments were performed: (i) The effects of acute volume overload on urine and serum cGMP concentrations and on urine volume and sodium output, and plasma protein concentration after 3 and 5 hr; and (ii) The effects of isatin on controls and on the acute volume overload-induced changes in blood and urine.

2.4. Statistical analysis

Statistical analysis of the data was made by analysis of variance (ANOVA). For significant ANOVA values, groups were compared using the Bonferroni adjustment for multiple comparisons, using GraphPad Prism software. This was also used for preparing the figure. A probability level of 0.05 was accepted as indicating significant differences.

3. Results

Fig. 1 shows that volume loading after 3 hr, as predicted, caused a large and highly significant increase in urinary output of cGMP. It also caused a large increase in total urinary volume and sodium output, and a reduction in plasma protein concentration. It caused a non-significant rise in plasma cGMP (Table 1).

Table 1 shows that isatin alone had a significant effect in reducing plasma protein concentration but no significant effect on the other parameters. However, after acute volume overload isatin had a significant normalising effect on both urine cGMP output and plasma protein levels (Table 1 and Fig. 1). Although it had no significant effect on plasma cGMP levels after fluid overload, the trend was in the same pattern as that for the urine. After 5 hr, all effects of isatin had disappeared.

4. Discussion

The model of fluid overload caused an increase in output of cGMP, urine volume, and total sodium content as expected (Table 1), and the fluid-induced increased output of

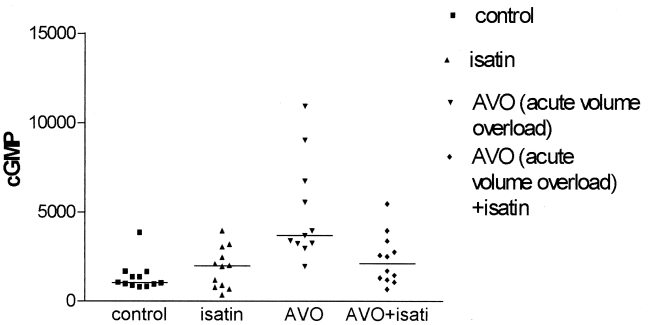

Fig. 1. The effects of fluid overload (AVO) and isatin (50 mg/kg i.p.) on the total output of cyclic GMP in urine after 3 hr. One-way ANOVA $P < 0.0001$. Control different from control + isatin $P < 0.01$; AVO different from AVO + isatin $P < 0.01$; both after Bonferroni correction.

cGMP was normalised by prior administration of isatin. This gives support to the *in vitro* findings concerning the effect of isatin on the ANP receptor. It is interesting to note that the pattern of change in plasma cGMP levels followed the urine cGMP levels, although the effects were not significant. It is possible that the effects of both overload and isatin were transient, and plasma levels had reverted towards baseline at the end of 3 hr. In contrast, the urine collection represents an integral over time and therefore shows the effects more clearly.

The effects of isatin on plasma protein concentration, presumably reflecting shifts in fluid between different compartments, are of interest. Under control conditions, isatin caused a dilution of plasma protein. This would be consistent with isatin acting in the opposite way from ANP, which is known to cause a shift in fluid balance from the vascular to the interstitial compartment [11]. However, after fluid overload, isatin helped to normalise the vascular plasma protein level.

The complexity of the response to isatin presumably reflects the complexity of the physiological situation. There are several receptor types for ANP (natriuretic peptide receptor [NPR]), NPR-A, NPR-B, and NPR-C), and there are also several natriuretic peptides (ANP, BNP, CNP, and urodilatin [12–14]. There may be different receptor subtypes in different tissues [15] and allosteric regulation by endogenous factors [16]. Both the response to fluid overload and to isatin may well reflect the different pattern of natriuretic peptides and receptors involved under different conditions.

Thus, in conclusion, we have shown that isatin can normalise fluid overload-induced increase in cGMP urinary output, and also plasma fluid balance. At doses of 50 mg/kg, it clearly has effects *in vivo*, consistent with it acting on the ANP receptor and inhibiting the generation of cGMP. In the absence of fluid overload, it tended to have opposite effects. It is intriguing that isatin is present endogenously in concentrations consistent with its *in vitro* actions. Whether the actions described here also occur physiologically or represent the effects of a pharmacological dose remains to be established.

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