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COMMENTARY

Isatin: A Link between Natriuretic Peptides and Monoamines?

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ABSTRACT. Isatin is an endogenous indole with a distinctive distribution in brain and tissues. In the brain, the highest levels have been found in the hippocampus (0.1 μg/g), and an immunocytochemical stain has shown specific localization within particular cells. *In vitro*, its most potent known actions are as an inhibitor of monoamine oxidase B (IC_{50} ~ 3 μM), and of atrial natriuretic peptide (ANP) receptor binding and ANP-induced guanylate cyclase (both with an IC_{50} ~ 0.4 μM). *In vivo*, isatin administration (10–200 mg/kg) causes an increase of monoamine neurotransmitter levels in the brain. Isatin is anxiogenic in animal models at doses of 10–20 mg/kg and sedative at higher doses. Its anxiogenic effects are unlikely to be due to inhibition of monoamine oxidase, but may possibly stem from interaction with the ANP system. Isatin may mediate a link between monoamines and the natriuretic peptide system, and its analogues may provide new pharmacological tools. BIOCHEM PHARMACOL 52;3:385–391, 1996.

KEY WORDS. ANP; monoamine oxidase; anxiety; stress; isatin

Isatin (2,3-dioxindole) is a bright orange-coloured compound with a long history and a broad range of pharmacological actions. It is present in the brain and other tissues at levels in which it is likely to have physiological effects. It can be both anxiogenic and sedative; it causes an increase in brain monoamine levels; it is an MAO¶ inhibitor, especially of MAO B; and it is a potent inhibitor of the ANP receptor. It was discovered early in the nineteenth century as an oxidation product of indigo [1, 2] and subsequently subjected to intensive scientific study (for reviews, see Refs. 3 and 4). Biologically, it was isolated in 1940 from the urine of rabbits fed with *o*-nitrophenylglyoxylic acid [5]. In 1988, it was identified in human urine and rat brain as a component of the endogenous MAO inhibitor tribulin [6, 7]. However, it now appears that most of the stress-related activity detected in rat brain and ascribed to tribulin stems from a selective MAO A inhibitory component or components, rather than isatin ([8–10] and unpublished observations).

At present, the only reliable way to quantify it is by gas chromatography–mass spectrometry [11], although an ELISA model has been developed for its assay in urine [12]. It is readily soluble in both water and ethyl acetate and passes easily through cell membranes. A dose of 50 or 100 mg/kg administered i.p., 30 min before killing the animal, resulted in a concentration of 9 mg/kg or about 60 μM, in rat brain [13].

TISSUE DISTRIBUTION

Isatin has a distinct and discontinuous distribution in rat brain and other tissues [9, 14, 15]. Its highest concentration appears to be in the vas deferens and seminal vesicles, and levels in the heart are somewhat higher than in the brain (Fig. 1). In the latter site, the hippocampus and cerebellum have the highest concentration (0.1 μg/g or about 1 μM), whereas the frontal cortex shows lower values. Such findings suggest that the compound has a particular function both in brain and in peripheral tissues. This assumption is supported further by recent studies with a specific immunohistochemical stain. Preliminary work shows isatin to be highly localized in the brain, with intense staining in particular structures. Figure 2 demonstrates its specific localization in the cerebellum (unpublished data). Other areas of intense staining in the brain include hippocampus, the lining of the ventricles, and the choroid plexus.

EFFECTS OF ISATIN ON MAO, MONOAMINES, AND THE ANP RECEPTOR

MAO

That isatin inhibits MAO has been known for many years [16]. More recently, it has been shown by several different

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¶ *Abbreviations:* ANP, atrial natriuretic peptide; AVP, arginine vasopressin; BNP, brain natriuretic peptide; CNP, C-type natriuretic peptide; MAO, monoamine oxidase; 5-HT, 5-hydroxytryptamine; and 5-HIAA, 5-hydroxyindoleacetic acid.

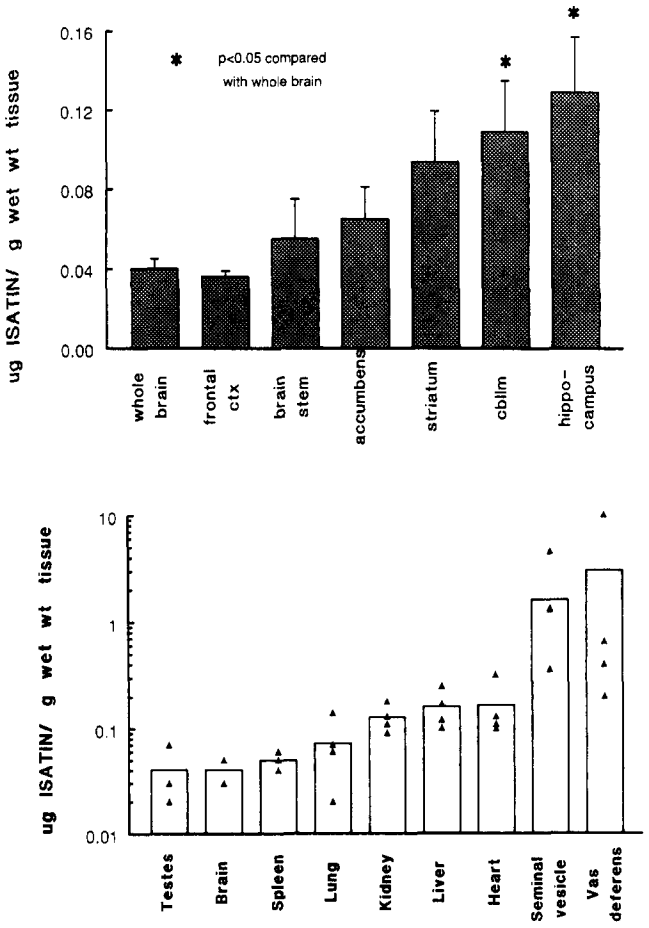

FIG. 1. Top panel: regional distribution of isatin in rat brain (means ± SEM, N = 4 sets of pooled tissues). Abbreviations: ctx, cortex; and cbllm, cerebellum. Bottom panel: regional distribution of isatin in rat tissues. Reprinted with permission of the publisher from Watkins P, Clow A, Glover V, Halket J, Przyborowska A and Sandler M, Isatin, regional distribution in rat brain and tissues. *Neurochem Int* 17: 321–323, Copyright 1990, by Elsevier Science Ltd. The Boulevard, Langford Lane, Kidlington OX5 1GB, U.K. [Ref. 14.]

groups to be a selective inhibitor of MAO B [6, 17, 18]. The inhibition is competitive, with an apparent inhibition constant for rat MAO B of 3–20 μM [6, 18, 19]. Inhibition of MAO A appears at higher concentrations, with a K_i value for the rat enzyme of 60–70 μM [6, 17]. The readily reversible, competitive mode of inhibition would explain the inability to observe this effect in tissue preparations after isatin administration to animals, as the homogenate is diluted in the assay mixture [20].

A large dose of isatin (200 mg/kg) 10 min after pretreatment of mice with the MAO inhibitor pargyline (50 mg/kg) has been shown to protect animals against a lethal dose of tryptamine [21]. The mechanism of this is not clear, but it may be that the large dose of the reversible inhibitor, isatin, masked and protected MAO against the action of the mechanism-activated irreversible inhibitor, pargyline.

Isatin, at high dosage (80–200 mg/kg), appears to be anticonvulsant in a variety of experimental preparations, including electroconvulsive shock [22], pentylene tetrazole-induced convulsions [16], and audiogenic seizures [23–26]. This may be connected with inhibition of MAO. In rats with inherited audiogenic epilepsy, the anticonvulsant effect of isatin was observed at a concentration of 80 mg/kg, and was comparable with that of the tight-bound short-acting MAO A inhibitor pirlindole [27, 28]. However, isatin causes sedation at higher dosage [29], and this may also play some part.

Monoamines

A consistent finding is that isatin, administered peripherally to rats in doses of 10 to 200 mg/kg, causes an increase in concentration of monoamine neurotransmitters in the brain (see Table 1). The nature of the effect depends on dose and time of exposure after isatin injection and, probably also, to the different rat strains used in various laboratories. At higher concentrations, both MAO A and B could be inhibited substantially, and, at first sight, this is a possible explanation for increased levels of noradrenaline and 5-HT, both of which are mainly metabolized by MAO A in rat brain. However, the effects are more complex and variable, even at high dosage, depending on brain region and monoamine being measured (see Table 1). In addition, most studies have found that an increase of 5-HT was not accompanied by a corresponding decrease in 5-HIAA, which would be expected if MAO were inhibited functionally [30–32]: 5-HIAA concentration, in fact, remained unchanged or was elevated slightly. There may be alternative mechanisms to account for an increase in monoamine levels, such as an increase of firing of monoamine neurones (which could also explain the anxiogenic effect of isatin), although there is no evidence for this at present.

In contrast with the whole brain, isatin did not influence the 5-HT and 5-HIAA content of the pineal gland [20]. It is of particular interest that the administration of high doses of isatin (50 and 200 mg/kg) had a substantially smaller effect on noradrenaline and 5-HT content of the brain of spontaneously hypertensive stroke-prone rats [32]. It is possible that there is a link here with the ANP system (see below).

ANP

Isatin causes potent inhibition of ANP binding to its receptor. A recent screening for the effect of 10 μM isatin on a wide range of different binding systems, performed by NovaScreen (Hanover, MD, U.S.A.), revealed that most of them were not affected [33]. Several (including potassium channel, low conductance Ca^{2+}, epidermal growth factor, and *N*-methyl-D-aspartate-4 receptor) were inhibited by 20–40%. The only receptor at which this concentration of isatin had a pronounced inhibitory effect (96%) was that for ANP. The IC_{50} value for such isatin inhibition was 0.4

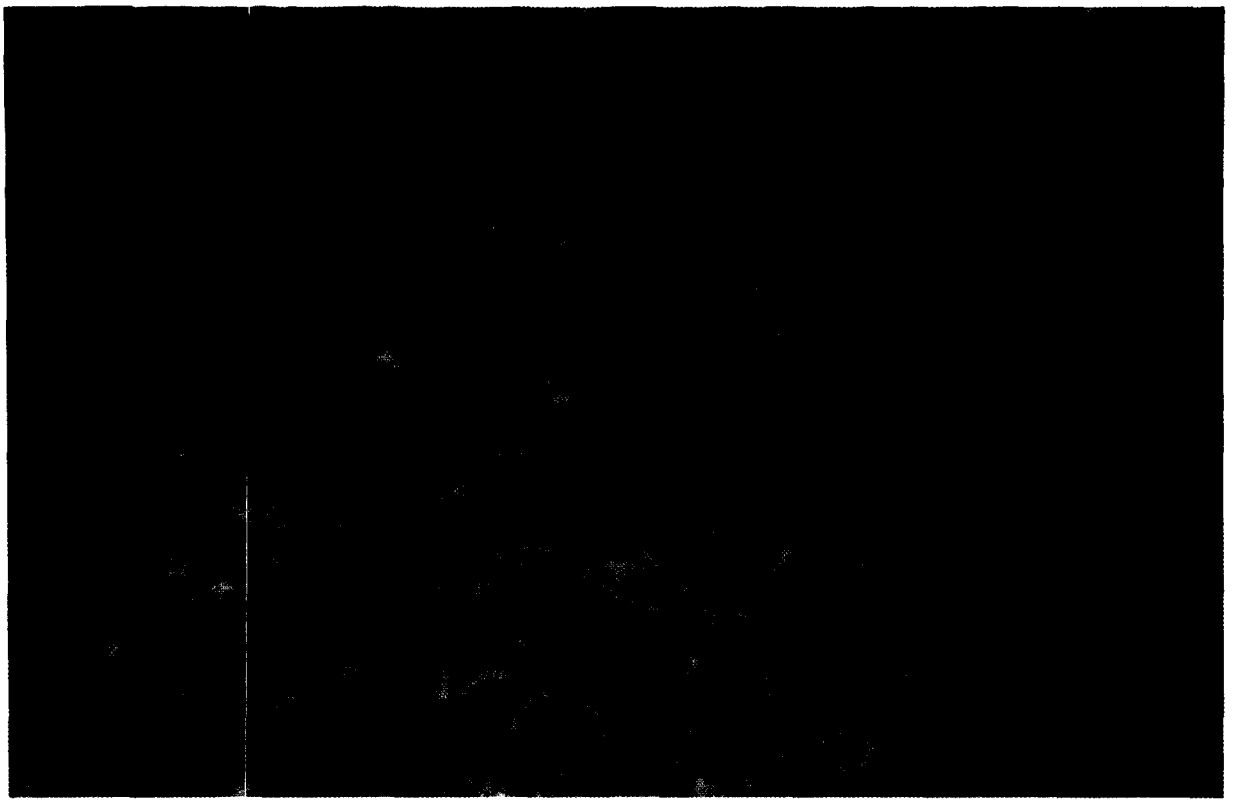

FIG. 2. Immunohistochemical localization of isatin-like activity in rat cerebellum, using a specific antibody raised against isatin (×10,800). The arrows show the specific pink staining of the Purkinje cells. Blue nuclear counterstaining with haematoxylin solution was also used. Blanks in which the primary antibody was replaced with buffer, or which used a primary antibody preadsorbed with isatin, showed no pink staining.

μM, one order of magnitude smaller than that of its effect on MAO B activity (IC_{50} ~ 4 μM). The effect of isatin on ANP binding was characterized by pronounced negative co-operativity (Hill coefficient = 0.28), suggesting a complex mechanism of inhibition rather than simple competition (Fig. 3).

Isatin has been shown to inhibit ANP-stimulated guanylate cyclase activity of rat brain, heart, and kidney membranes in a dose-dependent manner, reducing the formation of cyclic GMP [33]. Its effect was weaker when higher ANP concentrations were used. However, the interactions were not simply competitive.

The IC_{50} for the interaction between isatin and the ANP receptor is in the same concentration range as that found to be present in the brain (0.4 and 1 μM, respectively). This suggests that isatin may have a physiological role as well as pharmacological effects in this system.

ANXIOGENIC AND SEDATIVE EFFECTS

Isatin, at doses of 15–20 mg/kg, has been found to be anxiogenic in a range of rodent models such as the open field and elevated plus maze tests [29, 31], although not in all preparations used to test this type of behaviour [34]. These effects were similar to those induced by yohimbine or pentylene tetrazole [13]. Increasing the dose of isatin to 50 mg/kg and above has an opposite action, that of sedation, in both rodents and monkeys [31, 34]. The anxiogenic action of isatin in mice was attenuated by non-selective 5-HT or selective 5-HT_3 antagonists, by an antagonist of the dopamine D_2 receptor (pimozide), or by pretreatment of animals with the serotonergic neurotoxin 5,6-dihydroxytryptamine [31]. Fluoxetine, an inhibitor of 5-HT neuronal uptake, potentiated the anxiogenic effect of a suboptimal dose of isatin [31].

The anxiogenic agent pentylenetetrazole has been shown to cause an increase in rat and rabbit brain levels of isatin, but to have no effect on concentrations in rabbit liver [13, 35].

Bhattacharya and colleagues [36] have shown recently that ANP is anxiolytic in animal models, an effect that can be reversed by isatin at low (subanxiogenic) doses (10 mg/kg), but not by benzodiazepines. This finding provides the first evidence that isatin may act as a functional antagonist of ANP *in vivo*, although it does not prove that isatin is exerting its effects directly on the ANP receptor.

The anxiogenic effects of isatin were observed at doses that would not be expected to inhibit MAO A; MAO B inhibitors are neither anxiogenic nor sedative. Thus, some other mode of action must be involved to explain these effects of isatin on behaviour. It could have a direct effect on neuronal firing, which might also explain the increased

TABLE 1. Influence of isatin on neurotransmitter levels in the brain

Amine	Isatin dose (mg/kg)	Time of exposure (min)	Rat strain	Brain area	Effect (%)	Ref.
5-HT	80	60	Sprague-Dawley	Hypothalamus	+60	30
				Frontal cortex	+37	30
5-HT	80	120	Fischer 344N	Whole brain	+15	20
5-HT	10	30	Charles Foster albino	Whole brain	+33	31
	10	60		Whole brain	+77	31
	20	15		Whole brain	+96	31
	20	60		Whole brain	+97	31
Dopamine	20	15		Whole brain	+55	31
	20	30		Whole brain	+81	31
	20	60		Whole brain	+60	31
5-HT	50	120	Wistar Kyoto	Cortex	+47	34
				Hypothalamus	+18	34
	200	120		Cortex	+329	34
				Hypothalamus	+82	34
Noradrenaline	50	120		Cortex	+27	34
				Hypothalamus	−34	34
	200	120		Cortex	+428	34
				Hypothalamus	−46	34
5-HT	50	120	Stroke-prone spontaneously hypertensive	Cortex	+52	34
				Hypothalamus	+18	34
	200	120		Cortex	+93	34
				Hypothalamus	+22	34
Noradrenaline	50	120		Cortex	+11	34
				Hypothalamus	0	34
	200	120		Cortex	−5	34
				Hypothalamus	−16	34

levels of monoamine transmitter. It is also possible that its antagonist action at the ANP receptor is anxiogenic, acting by reversing the anxiolytic effect of ANP. ANP has been shown to inhibit the release of adrenaline and noradrenaline from the medulla [37]. ANP has also been shown to inhibit AVP release in the brain [38], and AVP is known to potentiate the release of both catecholamines and cortisol via its action at the ANP receptor. The mechanism for the sedative effects at higher dosage remains puzzling.

EFFECT ON WATER EXCRETION

The biological functions of natriuretic peptides are related to the regulation of sodium and water excretion and blood pressure [39]. They also antagonize both AVP and the renin–angiotensin–aldosterone system, inhibiting the secretion of renin and aldosterone. The discovery of an endogenous ligand that effectively influences specific ANP binding and inhibits intracellular signal transduction (cyclic GMP) at concentrations in which it is found physiologically, may represent a significant new *in vivo* mechanism for the regulation of water balance and the cardiovascular system.

Isatin administration to rats causes a reduction in daily urine excretion and its sodium content [40] (expressed per

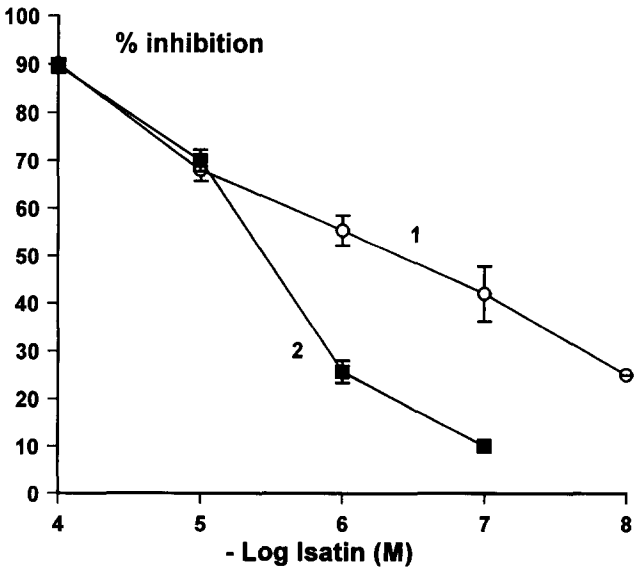

FIG. 3. Inhibition by isatin of [^{125}I]ANP binding (1) and MAO B activity (2). Results are the means ± SEM from 3–5 experiments. Reprinted with permission of the publisher from Glover V, Medvedev A and Sandler M, Isatin is a potent endogenous antagonist of guanylate cyclase-coupled atrial natriuretic peptide receptors. *Life Sci* 57: 2073–2079, Copyright 1995 by Elsevier Science Inc., 655 Avenue of the Americas, New York, NY 10010-5107, U.S.A. [Ref. 33.]

excreted volume). This finding points to a functional antagonism to the known endogenous ANP action on water balance. However, an "anxiogenic" dose of isatin (20 mg/kg, which influences the behaviour of experimental animals within 30 min) did not affect diuresis for the first 24 hr, its effect appearing only after 48 hr. A higher dose of isatin (50 mg/kg), which is sedative rather than anxiogenic, had a pronounced action on the 24-hr urine volume [40].

ANTI-INFECTION

Isatin, at higher doses, appears to be protective against certain types of infections or infestations. Symbiotic marine bacteria defend embryos of the shrimp, *Palaemon macrodactylus*, from a pathogenic fungus, *Lagenidum callinectes*, by producing isatin [41]. Bacteria-free embryos exposed to the fungus died, while similar embryos, re-inoculated with the bacteria or treated periodically with 1.4 mM isatin solution, survived. Isatin was also effective against the parasite *Echinococcus multilocularis* [42]. Isatin treatment of animals (jirds–*Meriones unguiculatus*) (50 mg/kg for 18 days) pre-infested with *E. multilocularis* caused a 27% reduction of parasite load, which was accompanied by a decrease of alkaline phosphatase and lactate dehydrogenase activities both in the parasite and in animal liver. These biochemical effects correlate with the known *in vitro* inhibition of rat alkaline phosphatase by isatin [43]. At millimolar concentrations, isatin has been found to inhibit several different enzymes, an effect that may contribute to its anti-infective action [15].

METABOLISM

Pathways for the synthesis and metabolism of isatin in animal tissues have not been established. One possible source would be via the action of the gut flora. It has been suggested [44] that dietary tryptophan may be converted into indole by the gut flora and then transported to the liver where it is oxidized. A comparison of the isatin content in urine of conventional and germ-free rats, which lack gut flora, showed that the latter was 50-fold lower than the former [45]. Thus, gut flora do have an important role in the production of urinary isatin in the rat. Although their role in humans is unknown, it may also be considerable. Isatin concentrations in brain and heart, however, were similar in germ-free and conventional animals, showing than an alternative method of synthesis must exist and also that the source of the urinary isatin does not contribute to the level in these tissues.

It has been suggested that isatin is also formed in tissues from phenylalanine or tryptophan [46]. While the isatin content of rat brain was unchanged after loading with tryptophan, phenylalanine, or tyrosine [13], preliminary experiments with rat brain homogenates showed that it can be formed *in vitro* from phenylalanine and tyrosine, but not from tryptophan (unpublished observations).

It is well known in organic chemistry that indole is readily oxidized by a variety of reagents. Thus, hydrogen peroxide converts it to indoxyl and then to indigo plus indirubin, a condensation product of indoxyl with isatin [47] (Fig. 4). Subsequent oxidization of isatin results in cleavage of the amide group of isatin with formation of anthranilic acid or isatinic acid.

In the only report in the literature on the metabolism of [^{14}C]indole administered to the rat, it was shown that isatin represented 5.8% of the 81% of ^{14}C-label excreted in the urine [48]. Aerobic incubation of indole with rat liver microsomes plus supernatant resulted in the formation of indoxyl, oxindole, *N*-formylanthranilic acid, anthranilic acid, indigotin, and indirubin. The occurrence of indigotin and indirubin in the incubation mixture suggests that indoxyl and isatin are formed under aerobic conditions [48].

In vitro, isatin can be destroyed easily by a high concentration of hydrogen peroxide [47, 49]. It is also readily metabolized by xanthine oxidase [49], producing hydrogen peroxide via the intermediate formation of superoxide [50]. Urate oxidase, which produces hydrogen peroxide without superoxide formation [50], was ineffective [49]. These results point to the involvement of superoxide in isatin metabolism *in vitro*; it remains to be seen whether this mechanism operates *in vivo*.

The sequential generation of four structurally related compounds, oxindole, isatin, dioxindole and anthranilic acid, was detected in micro-organisms denitrifying sewage sludge [46]. Isatin was produced as an intermediate when they were incubated with oxindole [51]. Thus, possible schemes for isatin synthesis from phenylalanine, via indoxyl, or from indole via oxindole, and metabolism to the end product, anthranilic acid, have been shown in isolated systems (Fig. 4). These pathways remain to be established in mammals, and the enzymes responsible for the sequence of chemical reactions are unknown.

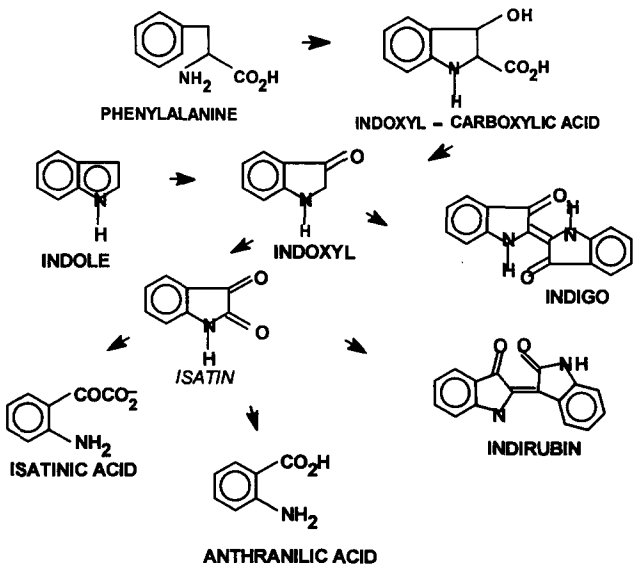

FIG. 4. Possible routes for the synthesis and degradation of isatin.

PHARMACOLOGICAL IMPLICATIONS

ANP is one of a family of natriuretic peptides. ANP and its related peptides, BNP and CNP, act on several different receptor systems [52]. These include at least two types, A and B, linked to the generation of cyclic GMP by activation of guanylate cyclase. They also act on a further site, type C or the clearance receptor, which is not coupled to guanylate cyclase [39]. ANP and BNP have similar biological profiles and are important in the potentiation of water and sodium excretion and the reduction of blood pressure; they also both inhibit the renin–angiotensin–aldosterone system [39]. ANP is present in high concentration in atrial tissue and at much lower levels in the brain; BNP is also present in high concentration in the heart; CNP is the most prevalent of the three in the central nervous system [53]. ANP is a potent vasorelaxant; CNP is also a strong vasorelaxant but has little diuretic or natriuretic action [39]. No other endogenous agonist, or antagonist, has been described for any of the natriuretic peptide receptors. There are several reports that some ANP analogues are capable of interfering with ANP binding and ANP-induced cyclic GMP accumulation [54, 55]. However, no evidence exists for their presence *in vivo*. There are also few selective pharmacological instruments for studying their mode of action. Morishita *et al.* [56] have described the isolation of an antagonist polysaccharide, HS-142-1, which is of microbial origin, and is a useful pharmacological tool [57]. When administered systemically, it has been shown to attenuate ANP-induced hypotension, diuresis, and natriuresis and to increase urinary cyclic GMP [58].

An endogenous antagonist, such as isatin, may have an important physiological or pathological role in counteracting the effects of the natriuretic peptides. If this is so, then methods that, in turn, reduce isatin levels may also be clinically valuable. Thus, isatin and its analogues (of which more than eighty have been synthesized in our laboratories) may be useful new tools in helping to unravel the complexities of the function of the different natriuretic peptides and their receptors.

Isatin is thus a compound with a distinct range of properties. It may provide a link between the control of monoamine neurotransmitter level, the control of mood, especially anxiety, and the natriuretic peptide system.

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