Biochemical Pharmacology, Vol. 58, pp. 1329–1334, 1999.

ISSN 0006-2952/99/$–see front matter
PII S0006-2952(9)00195-1

5-$HT_{2A/2C}$ Receptor Agonist-Induced Increase in Urinary Isatin Excretion in Rats

REVERSAL BY BOTH DIAZEPAM AND DEXAMETHASONE

Yumiko Tozawa,† Akira Ueki,‡ Tatsuo Shimosawa* and Toshiro Fujita**

*The Fourth Department of Internal Medicine, School of Medicine, University of Tokyo, Tokyo, 112-8688 Japan; and ‡Jichi Medical School, Ohmiya Medical Center, Saitama 330, Japan

ABSTRACT. Isatin, a stress-related biological substance, increases in rat urine in association with elevated catecholamine biosynthesis during stress. The goal of this study was to unravel how the biosynthetic pathway of isatin is related to stress response. The importance of the serotonergic compounds in anxiety, which is the major emotional process of stress response, has emerged. *m*-Chlorophenylpiperazine (*m*-CPP), a 5-$HT_{1A/1B/2A/2C}$ receptor agonist, and (±)-1-(4-iodo-2,5-dimethoxyphenyl)-2-aminopropane hydrochloride [(±)-DOI], a 5-$HT_{2A/2C}$ agonist, both of which have anxiogenic properties, induced a marked increase in 24-hr urinary isatin excretion, whereas neither 1-(*m*-chlorophenyl)-biguanide (*m*-CPBG), a 5-HT_3 agonist, nor 2-methyl-5-HT, a 5-$HT_{3,4}$ agonist, affected urinary isatin excretion. 5-$HT_{2A/2C}$ receptor antagonists such as ketanserin and ritanserin prevented the increase in urinary isatin excretion induced by the 5-$HT_{2A/2C}$ receptor agonist *m*-CPP. These findings are the first to provide evidence that pharmacological substances cause increases in urinary isatin excretion via specific 5-HT receptors, probably 5-$HT_{2A/2C}$ receptors. In addition, both the synthetic glucocorticoid dexamethasone and diazepam prevented the *m*-CPP-induced increase in urinary isatin excretion. These observations suggest that the mechanism by which *m*-CPP elicits enhancing effects on urinary isatin excretion has something in common with stress response involving activation of hypothalamic CRF cells and the sympathetic nervous system. BIOCHEM PHARMACOL **58**;8:1329–1334, 1999. © 1999 Elsevier Science Inc.

KEY WORDS. isatin; anxiety; 5-$HT_{2A/2C}$ receptor; *m*-CPP; dexamethasone; corticotropin-releasing factor

Isatin (indole-2,3-dione) is well known to have a broad range of pharmacological effects [1, 2]. Until it was identified as one component of tribulin [3], an endogenous monoamine oxidase inhibitory activity, isatin had not generated significant interest in relation to stress. Isatin has a discontinuous distribution in the brain and other tissues in rats, with markedly higher concentrations being detected in the vas deferens and seminal vesicles [1, 4]. There is some evidence from animal studies of a link between stress and isatin concentration in the brain [5]. More recently, 24-hr urinary isatin excretion was found to be elevated during stress [6]. The blockade of catecholamine biosynthesis completely abolishes the stress-induced increase in urinary isatin. This observation suggests that isatin production is stress-related and under noradrenergic control [6]. A pharmacological substance that triggers a "stress"-like response and also simultaneously induces an increase in isatin production is needed to unravel the stress-related biosynthesis of isatin.

The serotonergic compounds have emerged as playing key roles in anxiety, the major emotional process of stress response. 5-HT_{1A}, 5-HT_{2A}, and 5-HT_{2C} receptors have been suggested as being involved in anxiety. 5-HT_{2A} and 5-HT_{2C} receptors show great similarities not only in terms of second messengers and pharmacology but also in terms of molecular structure [7]. It is difficult in practice to distinguish the functions of 5-HT_{2A} and 5-HT_{2C} receptors.

In the last decade, several reports have been published indicating that *m*-CPP§ has anxiogenic effects in both animals and humans [8–13]. Although *m*-CPP, a 5-$HT_{1A/1B/2A/2C}$ agonist, is not truly selective for 5-HT_{2C} receptors, the antagonism of its effects by a variety of antagonists known to interact with 5-HT_{2C} receptors provides circumstantial support for the possible involvement of 5-HT_{2C} receptors [14]. *m*-CPP mimics the action of 5-HT to evoke hypothalamic CRF release, which is antagonized by ketanserin and ritanserin (5-$HT_{2A/2C}$ antagonists) [15]. In addition, activation of the hypophysis–pituitary–adrenal (HPA) axis due to *m*-CPP has been suggested to be primarily CRF dependent [16]. The proposed 5-HT_{2C}-mediated effects of *m*-CPP include hypophagia (also attributed to activation of central CRF type 2

† Corresponding author: Yumiko Tozawa, M.D., Ph.D., The Fourth Department of Internal Medicine, School of Medicine, University of Tokyo, 3-28-6 Mejirodai, Bunkyo-ku, Tokyo 112-8688, Japan. Tel. (81) 3-3943-1151, Ext. 396; FAX (81) 3-3814-1897.

Received 23 November 1998; accepted 25 January 1999.

§ *Abbreviations*: *m*-CPP, *m*-chlorophenylpiperazine; (±)-DOI, (±)-1-(4-iodo-2,5-dimethoxyphenyl)-2-aminopropane hydrochloride; *m*-CPBG, 1-(*m*-chlorophenyl)-biguanide; CRF, corticotropin-releasing factor; DEX, dexamethasone; AADC, aromatic amino acid decarboxylase; DOPA, 3-hydroxy-l-tyrosine; 5-HTP, 5-hydroxytryptophan; PVN, paraventricular nucleus; and SNS, sympathetic nervous system.

receptors) and penile erection (of psychozenic type; initiated by the supraspinal centers and attributed to lumbar sympathetic activation) [17].

Taken together, the molecular basis of the anxiogenic property of *m*-CPP may include activation of hypothalamic CRF secretion and sympathetic outflow, both of which play key roles in stress response. We expect that *m*-CPP is a candidate for a pharmacological substance that connects a quantitatively "stress"-like response with the increase in urinary isatin.

Previous studies have focused mainly on pharmacological or behavioral actions of exogenous isatin [18–21]. The anxiogenic action of exogenous isatin has been suggested to involve 5-HT and dopamine systems [22]. However, these findings do not necessarily reflect putative physiological actions of endogenous isatin. Thus, the putative physiological roles and the regulatory mechanism of isatin biosynthesis remain to be elucidated.

In an attempt to determine the regulatory mechanism of peripheral isatin production, especially in terms of stress response, we investigated whether a pharmacological substance, *m*-CPP, instead of a stressor, could increase urinary isatin excretion. The molecular basis of this mechanism may be related, at least in part, to that of stress response and can serve to unravel the stress-related biosynthesis of isatin.

MATERIALS AND METHODS

Subjects

All procedures were approved by The Animal Research Committee and met the Guidelines for the Care and Use of Laboratory Animals on the Hongo Campus of the School of Medicine, University of Tokyo.

Male Wistar rats (Japan Biological Materials Center), weighing 180–200 g upon arrival in our laboratory, were used in these experiments. The animals were housed in metabolic cages, had easy access to food pellets and tap water, and were maintained on a 12-hr light cycle. Rat urine samples were collected every day at 1:00 p.m. during the experiments.

Extraction of Isatin

Rat urine (1 mL) was diluted with 5 mL of distilled water and acidified with 6 M HCl to pH 1. Then the urine sample was heated for 10 min in a boiling water bath to solubilize the urine sediment. After cooling at room temperature, isatin was extracted with 10 mL of ethyl acetate. Next, the organic layer was evaporated under a stream of nitrogen; the residue was dissolved in 0.3 mL of methanol and then diluted with 5 mL of 50 mM potassium phosphate buffer, pH 7.4. Isatin was extracted using a disposable solid-phase column, the Mega Bond Elut C_{18} column (Varian), as described previously [23]. Each column was conditioned by washing with 6 mL of acetonitrile followed by washing with 6 mL of distilled water and 6 mL of 50 mM potassium phosphate buffer, pH 7.4. Then each sample was applied to the Mega Bond Elut C_{18} column, which was rinsed subsequently with 6 mL of 50 mM potassium phosphate buffer, pH 7.4, and 6 mL of distilled water; finally, the sample was eluted with 6 mL of 50 mM potassium phosphate buffer (pH 7.4):acetonitrile (85:15, v/v). The eluate was analyzed by HPLC.

HPLC Analyses

Reversed-phase HPLC analyses were performed using a Hitachi 655A chromatograph (Hitachi), as described previously [23]. Partial purification of isatin was carried out using a Shodex ES-502C column (100 × 7.6 mm i.d., 9.0 μm particle size; Showa Denko) under the following conditions: mobile phase, 50 mM potassium phosphate buffer (pH 7.4):acetonitrile (85:15, v/v); flow rate, 1.0 mL/min; 50°. The final HPLC analysis was carried out using a Kaseisorb LC ODS Super column (250 × 4.6 mm i.d., 5 μm particle size, and 120 Å pore size; Tokyo Chemical Industries). The column was equilibrated with 50 mM potassium phosphate buffer (pH 7.4):acetonitrile (85:15, v/v) at a flow rate of 1 mL/min. The fraction corresponding to isatin in the first-step analysis was injected directly onto the column. Separation was carried out at 50°, and the eluate was monitored by UV detection at 240 nm.

Drugs

All drugs were freshly prepared before use by dissolving in saline or 1% Tween 80 in saline and were administered by i.p. injection. Various 5-HT receptor agonists and antagonists, such as (±)-DOI, 2-methylserotonin maleate, tropisetron, methiothepin mesylate, LY-53857 maleate, *m*-CPBG, *m*-CPP dihydrochloride, ketanserin tartrate, and ritanserin, were obtained from the Funakoshi Pharmaceutical Co., Ltd. DEX (Wako Pure Chemical Industries) and diazepam (Sigma) also were prepared as mentioned above. HPLC-grade acetonitrile, methanol, and ethyl acetate were purchased from Wako Pure Chemical Industries. All other chemicals were of analytical grade.

Data Analysis

For the experiments on the effects of 5-HT receptor agonists on urinary isatin excretion, two-way ANOVA was used to determine the significance of changes in total isatin excretion as a function of time and treatment. For the experiments on the inhibitory effects of 5-HT_{2C} receptor antagonists on *m*-CPP-induced increase in urine isatin, the data from the *m*-CPP-injected group were subjected to one-way ANOVA with Bonferroni's test to determine the significance of the drug effect. The data from the ketanserin (ritanserin)-injected group were subjected to one-way ANOVA with Bonferroni's test to determine the significance of the dose effect. For the experiments on the effects of either DEX or diazepam in *m*-CPP-injected animals, data from vehicle-treated and DEX- or diazepam-treated animals

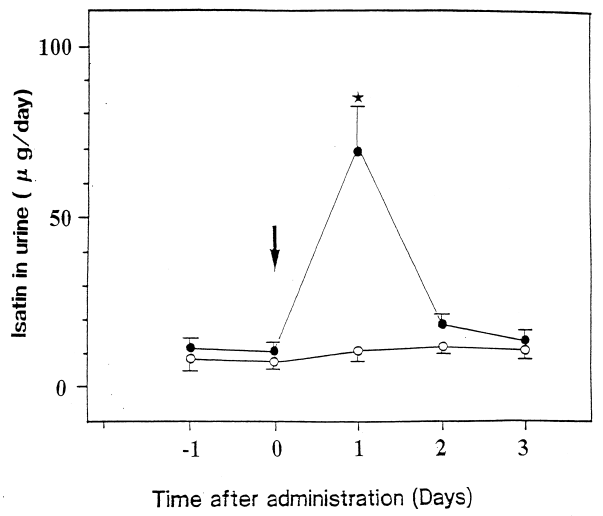

FIG. 1. Changes of the amount of isatin excreted into urine over a 24-hr period before and after administration of saline (○) and *m*-CPP (●). *m*-CPP dissolved in saline was administered i.p. at a dose of 0.1 mg/kg body weight. The arrow indicates *m*-CPP or saline administration. Each point represents the mean ± SEM of three rats. Key: (*) $P < 0.001$, compared with the saline group.

were compared, and statistical significance was calculated by Student's *t*-test.

RESULTS

Effects of m-CPP *on Urinary Isatin Excretion*

As shown in Fig. 1, urinary isatin excretion over a 24-hr period increased significantly in rats treated with *m*-CPP (0.1 mg/kg), while the amount of isatin excreted into urine over a 24-hr period did not change significantly in rats treated with saline. However, the increase in urinary isatin excretion was not apparent on day 2 following *m*-CPP administration.

Effects of Serotonergic Agonists on Urinary Isatin Excretion

Various doses (0.05 to 5.0 mg/kg) of *m*-CPP were administered to rats, and the 24-hr excretion of urinary isatin over a 24-hr period was determined before and after *m*-CPP i.p. administration. Moreover, we tested various serotonergic drugs to identify those that affected urinary isatin excretion. The data from the drug-injected group were subjected to two-way ANOVA. Among the serotonergic agonists tested, *m*-CPP (a 5-HT$_{1A/1B/2A/2C}$ receptor agonist) and (±)-DOI (a 5-HT$_{2A/2C}$ agonist) increased urinary isatin excretion (Table 1). The peak effect of *m*-CPP was observed at a dose of 0.1 mg/kg, although no significant concentration–effect relationship was found by ANOVA. On the other hand, *m*-CPBG (a 5-HT$_3$ agonist) and 2-methyl-5-HT (a 5-HT$_{3,4}$ agonist) did not affect urinary isatin excretion.

TABLE 1. Effects of 5-HT receptor agonists on urinary isatin output

5-HT agonists	Dose (mg/kg body wt)	Isatin in urine (μg/day)	
		Before administration	After administration
m-CPP	0.05	9.98 ± 1.41	23.74 ± 4.68*
	0.1	12.28 ± 0.48	81.96 ± 30.52*
	1.0	13.50 ± 2.55	61.89 ± 15.79*
	5.0	7.41 ± 2.16	60.32 ± 17.37*
(±)-DOI	0.05	10.42 ± 1.68	24.94 ± 3.01†
	0.1	11.32 ± 0.63	44.10 ± 9.66†
	1.0	10.57 ± 2.41	37.47 ± 7.72†
	5.0	12.44 ± 0.60	30.17 ± 1.83†
m-CPBG	0.1	12.10 ± 3.82	11.67 ± 2.25
	1.0	10.97 ± 2.06	10.53 ± 1.60
	5.0	8.70 ± 4.95	10.07 ± 2.55
	20.0	9.97 ± 3.59	9.27 ± 2.74
2-Methyl-5-HT	0.1	9.83 ± 1.29	9.33 ± 2.50
	1.0	11.17 ± 1.55	10.55 ± 0.57
	5.0	11.67 ± 3.22	9.0 ± 1.59
	20.0	9.77 ± 0.38	8.70 ± 1.73

Drugs were administered intraperitoneally. Each value represents the mean ± SEM of three rats.
*$P < 0.05$, compared with the values before administration (by ANOVA).
†$P < 0.025$, compared with the values before administration (by ANOVA).

Inhibitory Effects of 5-HT$_{2A/2C}$ Receptor Antagonists on the m-CPP*-Induced Increase in Urinary Isatin Excretion*

If urinary isatin excretion is mediated via 5-HT$_{2A/2C}$ receptors, 5-HT$_{2A/2C}$ receptor antagonists should suppress the *m*-CPP-induced increase in the urinary isatin excretion. Total isatin excretion during 24 hr of treatment with drugs or saline is shown in Fig. 2. Pretreatment of rats with ketanserin or ritanserin suppressed the *m*-CPP-induced increase in isatin excretion. The doses of ketanserin (0.01, 0.1, 1.0, and 5.0 mg/kg, i.p.) significantly reduced *m*-CPP-evoked isatin excretion, which was less at higher doses. Similarly, the doses of ritanserin (0.01, 0.1, 1.0, and 5.0 mg/kg, i.p.) significantly reduced *m*-CPP evoked isatin excretion, which also was less at higher doses. The data from the ketanserin- and the ritanserin-injected groups were subjected to one-way ANOVA with Bonferroni's test, and showed a significant dose effect ($P < 0.01$). These results support the hypothesis that urinary isatin excretion is mediated via 5-HT$_{2A/2C}$ receptors.

Effects of DEX on the m-CPP*-Induced Increase in Urinary Isatin Excretion*

In an attempt to determine whether CRF release in the PVN, the site of direct serotonergic synaptic input to CRF cells, plays a critical role in mediating the selective 5-HT receptor stimulation, we investigated the effects of DEX pretreatment on the *m*-CPP-induced increase in urinary isatin excretion.

Pretreatment with DEX (1 mg/kg, i.p.) prevented the *m*-CPP-induced increase in urinary isatin excretion significantly in comparison with vehicle pretreatment (Fig. 3).

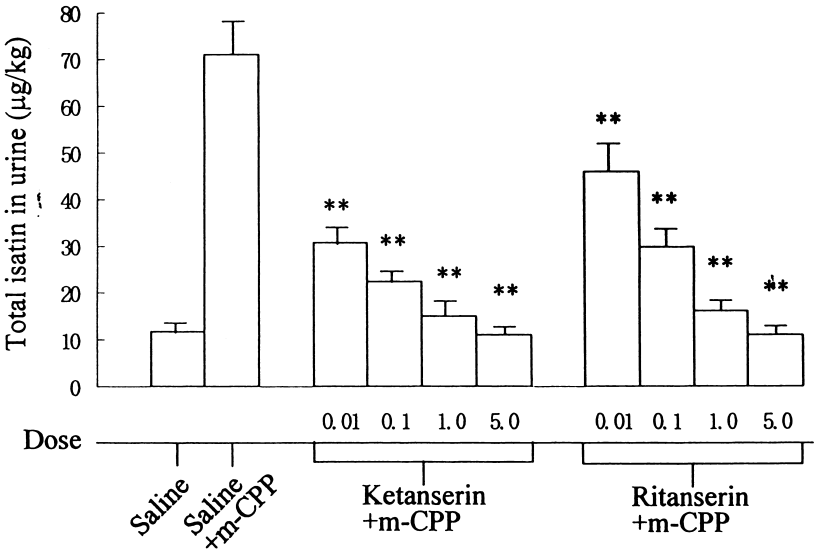

FIG. 2. Effects of two 5-HT$_{2C}$ receptor antagonists on the *m*-CPP-induced increase in urinary isatin excretion, compared with the vehicle (saline)-injected group. Ketanserin, ritanserin, or vehicle (saline) was administered i.p. 30 min before *m*-CPP (0.1 mg/kg, i.p.) treatment. Each value is the mean ± SEM of 5–10 determinations in each group. Key: (**) $P < 0.01$, compared with the saline- and *m*-CPP-injected group (Bonferroni's test).

Effects of Diazepam on the m*-CPP-induced Increase in Urinary Isatin Excretion*

A benzodiazepine drug, diazepam, is known to enhance γ-aminobutyric acid activity and to elicit its inhibitory effects on stress-induced activation of the SNS and CRF cells. The aim of this experiment was to determine whether the actions of *m*-CPP on urinary isatin excretion have something in common with stress response. Pretreatment with diazepam (1 mg/kg, i.p.) suppressed the *m*-CPP-induced increase in urinary isatin excretion in comparison with vehicle pretreatment (Fig. 4).

DISCUSSION

In the present study, we demonstrated that urinary isatin excretion in rats is modulated by 5-HT receptor agonists and antagonists. A 5-HT$_{1A/1B/2A/2C}$ receptor agonist, *m*-CPP, and a 5-HT$_{2A/2C}$ receptor agonist, (±)-DOI, increased urinary isatin excretion, whereas a 5-HT$_{3,4}$ receptor agonist, 2-methyl-5-HT, and a 5-HT$_3$ receptor agonist, *m*-CPBG, did not affect the isatin excretion values (Table 1). It should be noted that the doses of the 5-HT$_{2A/2C}$ antagonists ketanserin and ritanserin (0.01, 0.1, 1.0, and 5.0 mg/kg) significantly reduced *m*-CPP-evoked urinary isatin excretion, which was less at higher doses (Fig. 2). The blockade of 5-HT$_{2A/2C}$ receptors with selective 5-HT$_{2A/2C}$ receptor antagonists such as ketanserin, LY53857, and ritanserin tended to decrease urinary isatin excretion in rats without *m*-CPP treatment (data not shown). But the inhibitory potency of these 5-HT$_{2A/2C}$ antagonists on isatin excretion was not high enough to explain the complete blockade of the *m*-CPP-induced increase in urinary isatin excretion by these 5-HT$_{2A/2C}$ antagonists. For instance, the low doses of ritanserin (0.1 and 1.0 mg/kg), which when given alone did not decrease urinary isatin excretion,

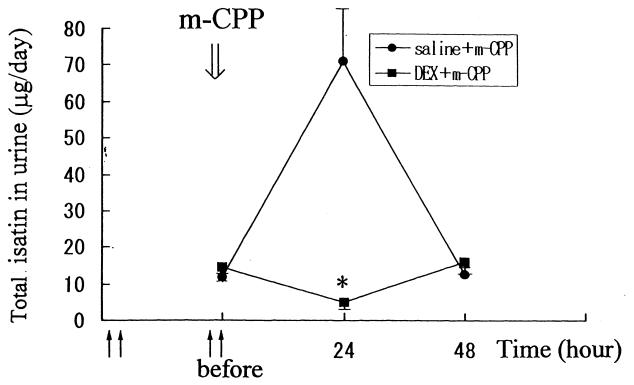

FIG. 3. Effect of DEX (1 mg/kg, i.p.) on the *m*-CPP-induced increase in urinary isatin excretion. *m*-CPP (0.1 mg/kg) dissolved in saline was administered i.p. Each value is the mean ± SEM of 3 determinations in each group. Key: (↑) DEX or saline injections; [⇓] *m*-CPP injection; (●) saline-pretreated and *m*-CPP-injected group; (■) DEX-pretreated and *m*-CPP-injected group; and (*) $P < 0.01$, compared with the saline-pretreated and *m*-CPP-injected group.

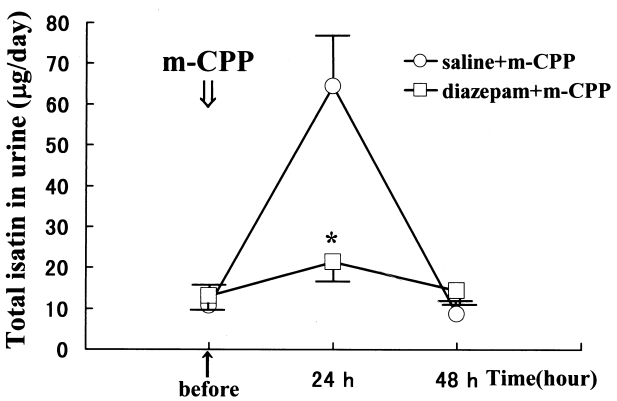

FIG. 4. Effect of diazepam (1 mg/kg, i.p.) on the *m*-CPP-induced-increase in urinary isatin excretion. *m*-CPP (0.1 mg/kg) was administered i.p. Each value is the mean ± SEM of 3 determinations in each group. Key: (↑) diazepam or saline injections; [⇓] *m*-CPP injection; and (*) $P < 0.01$, compared with the saline-pretreated and *m*-CPP-injected group.

produced a significant inhibition of the *m*-CPP-induced increase in urinary isatin excretion. Thus, it is strongly suggested that the enhancing effects of both *m*-CPP and (±)-DOI on urinary isatin are mediated by stimulation of 5-$HT_{2A/2C}$ receptors. Finally, isatin has been shown to be related not only to stressors but also to pharmacological substances through specific 5-HT receptors.

The goal of our study on isatin is to correlate isatin production to the stress response and to unravel the biosynthetic pathway and physiological role of isatin.

How can the mechanism by which *m*-CPP causes the increase in urinary isatin excretion be connected with "stress response"?

CRF, which stimulates primarily pituitary ACTH secretion, is thought to be an important integrative factor capable of coordinating endocrine, autonomic, and behavioral responses to stress [24–26]. CRF and the central noradrenergic systems participate in a positive feedback loop, with each system reinforcing the function of the other. The peripheral "limb" of the latter is known as the SNS, which is also activated directly by the central CRF. Thus, both the CRF and the SNS play essential roles in stress response.

In the present study, pretreatment with DEX (1 mg/kg) completely abolished the *m*-CPP-induced increase in urinary isatin excretion. This dose of DEX has been reported to suppress CRF mRNA synthesis and CRF release in the PVN [27, 28]. The *in vivo* action of *m*-CPP to evoke pituitary ACTH secretion via CRF release was abolished completely by DEX in the previous study. *m*-CPP mimics the action of 5-HT *in vitro* to evoke hypothalamic CRF release, which is antagonized by the 5-$HT_{1/2}$ receptor antagonist metergoline and, with similar potency, by the 5-$HT_{2A/2C}$ receptor antagonists ketanserin and ritanserin.

Furthermore, by acting directly at 5-HT_{2C} receptors, *m*-CPP is suggested to cause hypophagia, which has been attributed recently to the activation of CRF type 2 receptors. The CRF cells in the PVN receive direct serotonergic input via 5-HT_{2C} receptors. The stress-induced increase in urinary isatin excretion was also abolished by 0.5 to 2.0 mg/kg doses of DEX. Thus, *m*-CPP may employ a mechanism similar to that hypothesized in the case of stress for increasing urinary isatin excretion, including enhanced CRF secretion in the PVN.

The observation in the present study that diazepam also suppressed *m*-CPP-induced isatin excretion supports this hypothesis because diazepam completely abolishes the stress-induced increase in urinary isatin excretion [6].

A recent study has shown that activation of catecholamine biosynthesis is essential to the stress-induced increase in urinary isatin [6]. The stress-induced increase in urinary isatin excretion may be based on the activation of the SNS. The proposed 5-HT_{2C}–mediated effects of *m*-CPP include penile erection. Because 5-HT_{2C} receptors are heavily localized in the CNS and mediate direct serotonergic input to the CRF cells in the PVN, *m*-CPP may evoke lumbar sympathetic activation leading to psychogenic-type penile erection through a centrally controlled mechanism. Also, it should be noted that isatin distributes into rat tissues richly innervated by the sympathetic nerves, with the highest concentrations being detected in the vas deferens and seminal vesicles. Sympathetically mediated smooth muscle contraction of the vas deferens and seminal vesicles initiates the third phase of the sexual response in the male. Although a specific neurotransmitter pathway recruited by *m*-CPP remains to be elucidated, the enhancing effect of *m*-CPP could include the activation of the SNS.

In fact, central administration of CRF is well known to stimulate sympathetic outflow and to induce anxiety. Most physical symptoms caused by anxiety are closely related to sympathetic activation. Further substantial evidence is needed to establish what neurotransmitter pathway *m*-CPP employs to elicit its anxiogenic action. *m*-CPP may evoke a "stress-like" response in the CNS.

The biosynthetic pathway of isatin in mammals has remained unclear thus far, despite extensive studies. One candidate pathway is a constituent of oxidative pathways of *l*-tryptophan. This idea may generate much interest in terms of a putative mutual regulatory action among the aromatic amino acids to form neurotransmitters that play key roles in stress response. Two points should be stressed primarily. First, AADC, commonly employed to decarboxylate both DOPA and 5-HTP, has a low K_m and a high V_{max} with respect to DOPA. In addition, AADC does not appear to be modulated by the activation of sympathetic neurons that increase mRNA for tyrosine hydroxylase and dopamine β-hydroxylase. Thus, in a rapid activation of the SNS, possible saturation of AADC with a large amount of DOPA may result in the blockage of 5-HTP synthesizing pathway through 5-HTP. This may shift tryptophan metabolism to an alternative oxidative pathway such as the kynurenine pathway. The facts that the systemic administration of *m*-CPP induces decreased 5-HT metabolism and that acute food deprivation causes a significant decrease in the levels of tryptophan and 5-HT may provide circumstantial support to the idea. Second, oxidative *l*-tryptophan metabolism, besides modulating to the formation of 5-HT via 5-HTP, is known to generate various kinds of oxidative metabolites such as kynurenine and anthranilate. Some of them have potent antioxidant properties and serve as a local antioxidant defense during oxidative stresses such as infections. The synthetic pathway of isatin via indole-3-acetic acid towards anthranilic acid has been described in bacteria [29]. The idea that isatin is one constituent of these oxidative *l*-tryptophan metabolites may be well supported by the observation that isatin has a potent antifungal action.

The levels of these antioxidants are subjected to enzymatic up- and down-regulation in response to oxidative stress. Among these, indoleamine-2,3-dioxygenase, which is the rate-limiting enzyme in *l*-tryptophan catabolism in mammalian cells, is induced strongly by interferon-γ and interleukin-1 as well as by oxidative stress, leading to

markedly elevated levels of oxidative compounds along the kynurenine pathway both centrally and peripherally during stress [30]. Finally, it can be hypothesized that some kinds of stress cause an increase of interleukin-1 and a shift of *l*-tryptophan metabolism towards overproduction of oxidative metabolites, including isatin, rather than towards 5-HT synthesis.

In conclusion, a pharmacological substance, *m*-CPP, can induce a marked increase in urinary isatin excretion. The action of *m*-CPP may implicate the activation of CRF secretion and of sympathetic neurons. Considering the recent report on the involvement of the SNS in isatin production [6], elevated DOPA derived from the sympathetic neurons may promote the change in *l*-tryptophan metabolism during stress, leading to the increase in isatin production.

References

1. Glover V, Bhattacharya SK and Sandler M, Isatin—a new biological factor. *Indian J Exp Biol* **29**: 1–5, 1991.
2. Medvedev AE, Clow A, Sandler M and Glover V, Isatin: A link between natriuretic peptides and monoamines? *Biochem Pharmacol* **52**: 385–391, 1996.
3. Glover V, Halket JM, Watkins PJ, Clow A, Goodwin BL and Sandler M, Isatin: Identity with the purified endogenous monoamine oxidase inhibitor tribulin. *J Neurochem* **51**: 656–659, 1988.
4. Watkins P, Clow A, Glover V, Halket J, Przyborowska A and Sandler M, Isatin, regional distribution in rat brain and tissues. *Neurochem Int* **17**: 321–333, 1990.
5. Bhattacharya SK, Clow A, Przyborowska A, Halket J, Glover V and Sandler M, Effect of aromatic acids, pentylenetetrazole and yohimbine on isatin and tribulin activity in rat brain. *Neurosci Lett* **132**: 44–46, 1991.
6. Tozawa Y, Ueki A, Manabe S and Matsushima K, Stress-induced increase in urinary isatin excretion in rats. Reversal by both dexamethasone and α-methyl-*p*-tyrosine. *Biochem Pharmacol* **56**: 1041–1046, 1998.
7. Pritchett DB, Back AWJ, Wozny M, Taleb O, Daltoso R, Shih JC and Seeburg PH, Structure and functional expression of cloned rat serotonin 5-HT_2 receptor. *EMBO J* **7**: H35–H40, 1988.
8. Mueller EA, Murphy DL and Sunderland T, Neuroendocrine effects of *m*-chlorophenylpiperazine, a serotonergic agonist, in humans. *J Clin Endocrinol Metab* **61**: 1179–1184, 1985.
9. Charney DS, Woods SW, Goodman WK and Heninger GR, Serotonin function in anxiety. II. Effects of the serotonin agonist MCPP in panic disorder patients and healthy subjects. *Psychopharmacology* **92**: 14–24, 1987.
10. Kahn RS, Asnis GM, Wetzler S and von Praag HM, Neuroendocrine evidence for serotonin receptor hypersensitivity in panic disorder. *Psychopharmacology* **96**: 360–364, 1988.
11. Klein E, Zoher J, Geraci MF, Murphy DL and Uhde TW, Anxiogenic effects of *m*-CPP in patients with panic disorder: Comparison to caffeine's anxiogenic effects. *Biol Psychiatry* **30**: 973–984, 1991.
12. Germine M, Goddard AW, Woods SW, Charney DS and Heninger GR, Anger and anxiety responses to *m*-chlorophenylpiperazine in generalized anxiety disorder. *Biol Psychiatry* **32**: 457–461, 1992.
13. Kennett GA, Whitton P, Shan K and Curzon G, Anxiogenic-like effects of mCPP and TFMPP in animal models are opposed by 5-HT_{1C} receptor antagonists. *Eur J Pharmacol* **164**: 445–454, 1989.
14. Hoyer D, Clarke DE, Fozard JR, Hartig PR, Martin GR, Mylecharane EJ, Saxena PR and Humphrey PP, International Union of Pharmacology classification of receptors for 5-hydroxytryptamine (Serotonin). *Pharmacol Rev* **46**: 157–203, 1994.
15. Calogero AE, Bernardini R, Margioris AN, Bagdy G, Gallucci WT, Munson PJ, Jamarkin L, Tomai TP, Brady L, Gold PW and Chrousos GP, Effects of serotonergic agonists and antagonists on corticotropin releasing hormone secretion by explanted rat hypothalami. *Peptides* **10**: 189–200, 1989.
16. Calogero AE, Bagdy G, Szemeredi K, Tartaglia ME, Gold PW and Chrousos GP, Mechanisms of serotonin receptor agonist-induced activation of the hypothalamic–pituitary–adrenal axis in the rat. *Endocrinology* **126**: 1888–1894, 1990.
17. Keck W, Jackson A and Colpaert FC, Behavioral pharmacology of antagonists at 5-HT_2/5-HT_{1C} receptors. *Neurosci Behav Rev* **16**: 95–105, 1992.
18. Morley JE, Farr SA and Flood JF, Isatin inhibits food intake in mice. *Eur J Pharmacol* **305**: 23–29, 1996.
19. Sandler M, Clow A, Watkins PJ and Glover V, Tribulin—an endocoid marker for anxiety in man. *Stress Med* **4**: 215–219, 1988.
20. Clow A, Glover V, Sandler M and Tiller J, Increased urinary tribulin output in generalized anxiety disorder. *Psychopharmacology* **95**: 378–380, 1988.
21. Sandler M, Przyborowska A, Halket J, Watkins P, Glover V and Coates ME, Urinary but not brain isatin levels are reduced in germ-free rats. *J Neurochem* **57**: 1074–1075, 1991.
22. Bhattacharya SK and Acharya SB, Further investigations on the anxiogenic action of isatin. *Biogen Amines* **9**: 453–463, 1993.
23. Manabe S, Gao Q, Yuan J, Takahashi T and Ueki A, Determination of isatin in urine and plasma by high-performance liquid chromatography. *J Chromatogr B Biomed Sci Appl* **691**: 197–202, 997.
24. Rivier CL and Plotsky PM, Mediation by corticotropin releasing factor (CRF) of adenohypophysial hormone secretion. *Annu Rev Physiol* **48**: 475–494, 1986.
25. Sutton RE, Koof GF, LeMoal M, Rivier J and Vale W, Corticotropin-releasing factor produces behavioral activation in rats. *Nature* **297**: 331–333, 1982.
26. Fisher LA and Brown MR, Central regulation of stress responses: Regulation of the autonomic nervous system and visceral function by corticotrophin releasing factor-41. *Baillieres Clin Endocrinol Metab* **5**: 35–50, 1991.
27. Suda T, Yajima F, Tomori N, Demura H and Shizume K, *In vitro* study of immunoreactive corticotropin-releasing factor release from the rat hypothalamus. *Life Sci* **37**: 1499–1505, 1985.
28. Imaki T, Nahan JL, River C, Sawchenko PE and Vale W, Differential regulation of corticotropin-releasing factor mRNA in rat brain regions by glucocorticoids and stress. *J Neurosci* **11**: 585–599, 1991.
29. Jensen JB, Egsgaard H, Onckelen HV and Jochimsen BU, Catabolism of indole-3-acetic acid and 4- and 5-chloroindole-3-acetic acid in *Bradyrhizobium japonicum*. *J Bacteriol* **177**: 5762–5766, 1995.
30. Christen S, Peterhans E and Stocker R, Antioxidant activities of some tryptophan metabolites: Possible implication for inflammatory diseases. *Proc Natl Acad Sci USA* **87**: 2506–2510, 1990.