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Stress-induced Increase in Urinary Isatin Excretion in Rats

REVERSAL BY BOTH DEXAMETHASONE AND α-METHYL-*P*-TYROSINE

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ABSTRACT. The effects of acute food deprivation and acute cold exposure on 24-hr urinary isatin excretion in rats and a mechanism responsible for changes in urinary isatin excretion during stress were investigated. This is the first study to demonstrate by HPLC that urinary isatin excretion is increased by stress. Both types of stress induced a marked increase in urinary isatin excretion during the 24 hr following the initiation of stress. Dexamethasone administration prevented the increase in urinary isatin excretion induced by both of the different types of stress. Furthermore, administration of either the benzodiazepine receptor agonist diazepam or the tyrosine hydroxylase inhibitor α-methyl-*p*-tyrosine prevented the increase in urinary isatin excretion induced by acute food deprivation, whereas the dopamine-β-hydroxylase inhibitor diethyldithiocarbamate proved ineffective. These observations suggest that during stress, activated catecholamine-synthesizing cells and corticotropin-releasing factor cells, both of which play central roles in stress responses, may be involved in total isatin production. Isatin may serve as an endogenously generated marker for some types of stress. BIOCHEM PHARMACOL **56**;8:1041–1046, 1998. © 1998 Elsevier Science Inc.

KEY WORDS. isatin; rat; food deprivation; cold exposure; dexamethasone; α-methyl-*p*-tyrosine

Isatin (indole-2,3-dione) has a wide range of pharmacological effects including the induction of arousal, a reduction in the duration of slow-wave sleep, an increase in the seizure threshold in rats, and a decrease in food and water intake in mice [1–5].

Isatin has been identified as one component of tribulin [6], an endogenous MAO§ inhibitor [7]. Tribulin activity has been shown to be related to severe cold restraint [6, 8] or chemically induced stress in animals, and also has been shown to be augmented significantly in pathological conditions in humans, such as panic disorders, generalized anxiety disorders, mania, and agitated depression [9]. Further precise investigations have shown isatin to act *in vitro* as an inhibitor of MAO-B and atrial natriuretic peptide receptor binding [10]. It should be noted that isatin is anxiogenic in animals at doses of 10–20 mg/kg [11] and is sedative at higher doses [1]. Isatin is distributed discontinuously in the brain and other tissues in rats, with the highest concentrations in the brain being detected in the hippocampus [1, 12]. Isatin may be involved in some behavioral and emotional responses to stress and have a physiological role.

There is some evidence from animal studies of a link between stress and isatin concentration in the brain [13]. However, in previous studies, the observation that urinary isatin concentrations do not correlate with feelings of stress or general arousal, which correlate with endogenous MAO-B inhibitory activity, casts doubt on the suggestion that isatin is the major MAO-B inhibitory component in tribulin [14]. Despite extensive investigations of neurochemical and behavioral changes related to isatin [5, 15–17], the neurochemical basis of its anxiogenic properties and the meaning of urinary isatin levels in terms of the central or peripheral neural function remain to be elucidated.

The response of an organism to stress usually involves activation of both the HPA axis and the sympathetic-adrenomedullary system. CRF is one of the best known stress-related neuropeptides. In addition to activating the HPA axis, CRF exhibits direct neurotropic actions. CRF may activate the noradrenergic system in restricted regions of the CNS, such as the locus coeruleus, prefrontal cortex, hypothalamus, and hippocampus [18, 19], leading to many stress responses. Furthermore, CRF acts to stimulate sympathetic outflow. We expect that the production and excretion of isatin in the periphery may be regulated by a centrally controlled mechanism involving the activation of CRF neurons and the noradrenergic neurons. Previous

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§ *Abbreviations:* α-MT, α-methyl-*p*-tyrosine; CRF, corticotropin releasing factor; DA, dopamine; DDC, diethyldithiocarbamate; DEX, dexamethasone; DOPAC, dihydrdoxyphenylacetic acid; HPA, hypothalamo-pituitary-adrenocortical; MAO, monoamine oxidase; NA, noradrenaline; PVN, paraventricular nucleus; SNS, sympathetic nervous system; and TH, tyrosine hydroxylase.

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studies have described that 2- and 1.5-hr cold restraint stresses acutely increase tribulin in rat urine and in rat heart and kidney, respectively [8, 20]. Cold exposure is well known to activate the SNS, which richly innervates heart and kidneys. Tribulin, as well as isatin, has a characteristic distribution in rat tissues, with markedly high levels being detected in the superior cervical ganglion, vas deferens, seminar vesicles, heart, and kidney, all of which are richly innervated sympathetically [12, 20]. These observations strongly suggest that the increased sympathetic activity under stress results in the increase in tribulin. It has been well known that fasting for more than 48 hr suppresses, and acute cold stress stimulates, the SNS in rodents. In addition, fasting has been suggested to activate an ascending adrenergic projection that stimulates CRF release during 48 hr [21]. The synthetic glucocorticoid DEX reduces the production and release of CRF in the PVN [22–25] and of adrenocorticotropic hormone. In the present study, we measured 24-hr urinary isatin excretion in rats exposed to different types of stress (72-hr food deprivation or 2-hr cold exposure) and investigated the effects of DEX, the benzodiazepine receptor agonist diazepam, and the TH inhibitor α-MT on the increase in urinary isatin excretion observed during stress, with a view to determining whether increased urinary isatin excretion is associated with CRF neuron activation and involves the activation of catecholaminergic pathways.

MATERIALS AND METHODS

Animals

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

For all studies, male Wistar rats (180–200 g) obtained from the Japan Biological Materials Center were used and housed, one per metabolic cage, in a temperature-controlled room (23°) (except during exposure to cold when cages were placed in a cold room at 4°) under a 12:12-hr light–dark cycle. Animals were allowed free access to water and standard laboratory chow except where stated. While fasting, the animals were permitted free access to water. Rat urine samples were collected every 24 hr at 1:00 p.m. throughout the study.

Extraction of Isatin

Rat urine (1 mL) was diluted with 5 mL of distilled water and acidified with 6 N HCl to pH 1. The urine sample was heated for 10 min in a water bath (≧ 95°) and then cooled at room temperature. Using 10 mL of ethyl acetate, isatin was extracted into the organic layer. Next, the organic layer was evaporated under a stream of nitrogen, and 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 [26]. 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. Each sample was applied onto the Mega Bond Elut C_{18} column, which was rinsed with 6 mL of 50 mM potassium phosphate buffer, pH 7.4, and 6 mL of distilled water, and then 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. The compound purified with HPLC was confirmed to be isatin from its absorbance and mass spectrum.

HPLC Analysis

Reversed-phase HPLC analysis was performed using a Hitachi 655A chromatograph (Hitachi) as described previously [26]. The 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; 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 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

The following chemicals and drugs were used: DEX (Wako Pure Chemical Industries), diazepam (Sigma Chemicals), α-MT and DDC (Wako Pure Chemical Industries). All drugs were dissolved just prior to use in saline or 1% Tween 80 in saline, and then administered i.p. HPLC-grade acetonitrile, methanol, and ethyl acetate were obtained from Wako Pure Chemical Industries. All other chemicals were of analytical grade.

Data Analysis

For experiments on the stress-induced increase in isatin, data from pre-stressed and stressed (at 24, 48, or 72 hr) animals were compared, and statistical significance was calculated by Student's paired *t*-test. No significance was found between data from pre-stressed and non-stressed (control) animals. For the experiments on the effects of DEX in cold-exposed animals and on the effects of diazepam or α-MT in food-deprived animals, data from vehicle-injected and each group of drug-injected animals were compared. Statistical significance was calculated by Student's *t*-test. For the experiments on the effects of two doses of DEX in food-deprived animals, ANOVA, with Bonfer-

roni's test for multiple comparisons, was used. Data from non-stressed and α-MT-injected animals were shown for comparison. A group of vehicle-injected rats was kept and assayed together with the drug-injected rats. Data from each group of drug-injected animals were shown in a separate figure in comparison with vehicle-injected animals.

RESULTS

Effect of Acute Food Deprivation and Acute Cold Exposure on Urinary Isatin Excretion

To investigate the effects of states of stress in rats, total urinary isatin excretion was measured in 24-hr urine samples before and after acute food deprivation and acute cold exposure. Both acute food deprivation (Fig. 1A) and acute cold exposure (Fig. 1B) induced a sharp increase in urinary isatin excretion during the 24 hr following the initiation of stress, after which the values fell below pre-stress values.

Influence of Pretreatment with DEX on the Acute Food Deprivation-induced Increase in Urinary Isatin Excretion

We assessed the involvement of CRF in the food deprivation-induced increase in urinary isatin excretion. The high-dose (2 mg/kg/day) DEX-pretreated rat group showed 93% suppression of urinary isatin excretion relative to the vehicle control group and 67% suppression compared with the pre-fasting level (Fig. 2), whereas the DEX-treated rat group free of stresses exhibited unchanged levels of urinary isatin excretion compared with the vehicle control group (data not shown). High-dose DEX (2 mg/kg/day) was found to tend to inhibit the stress-induced increase in urinary isatin excretion more strongly than low-dose DEX (0.5 mg/kg/day) (Fig. 2), although no significance was found in a comparison of data from the two doses of DEX.

Influence of Pretreatment with DEX on the Acute Cold Exposure-induced Increase in Urinary Isatin Excretion

The aim of this experiment was to determine whether the elevation in urinary isatin excretion following acute cold exposure was mediated by a mechanism similar to that involved in changes in urinary isatin excretion during acute food deprivation. In the rat group pretreated with a high dose (2 mg/kg/day) of DEX, the increase in urinary isatin excretion was suppressed markedly compared with that observed in the saline-pretreated rat group (Fig. 3).

Influence of Pretreatment with Diazepam on Urinary Isatin Excretion Observed during Food Deprivation

The benzodiazepine receptor agonist diazepam is a well known anxiolytic agent that antagonizes the action of CRF. We found that diazepam (1 mg/kg) prevented the increase in urinary isatin excretion induced by acute food deprivation (Fig. 4).

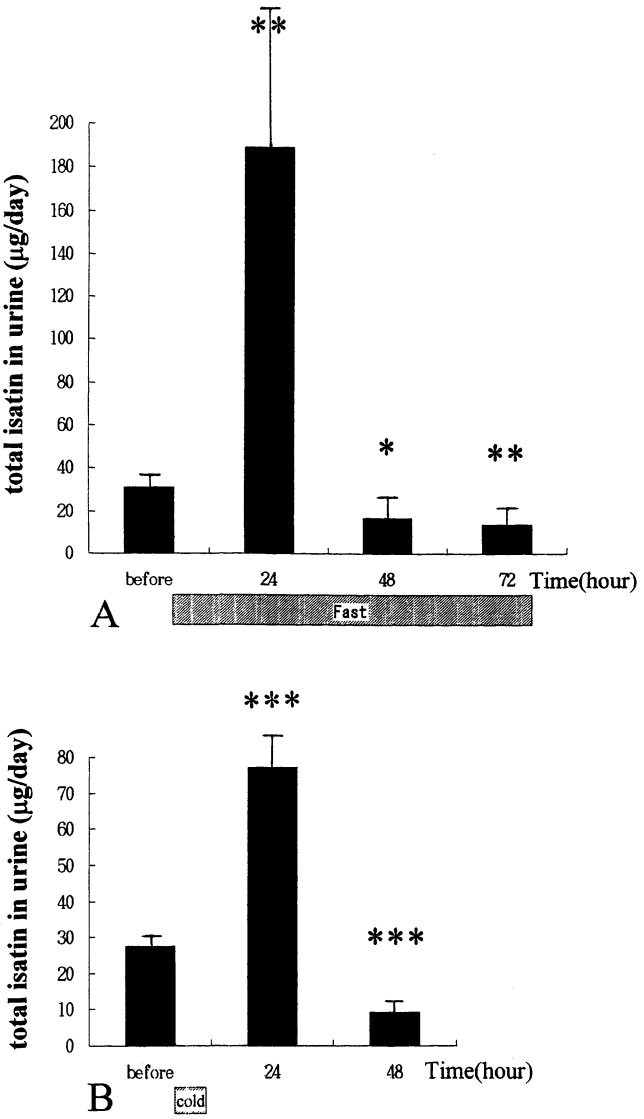

FIG 1. Effects of food deprivation (A) and cold exposure (B) on 24-hr urinary isatin excretion. Urine for analysis "before" stress was collected over a 24-hr period (1:00 a.m. to 1:00 p.m.) just before the initiation of stress. Each value is the mean ± SEM of 5–6 determinations in each group. Key: (*) $P < 0.025$, (**) $P < 0.005$, (***) $P < 0.0005$, compared with the value before stress.

Influence of Pretreatment with α-MT or DDC on Urinary Isatin Excretion Observed during Food Deprivation

The aim of this experiment was to investigate the possible involvement of the catecholamine synthetic pathway in the augmentation of urinary isatin excretion. α-MT (300 mg/kg), a potent inhibitor of TH which catalyzes the rate-limiting step in NA biosynthesis, completely prevented the increase in urinary isatin excretion induced by acute food deprivation (Fig. 5), whereas DDC (500 mg/kg),

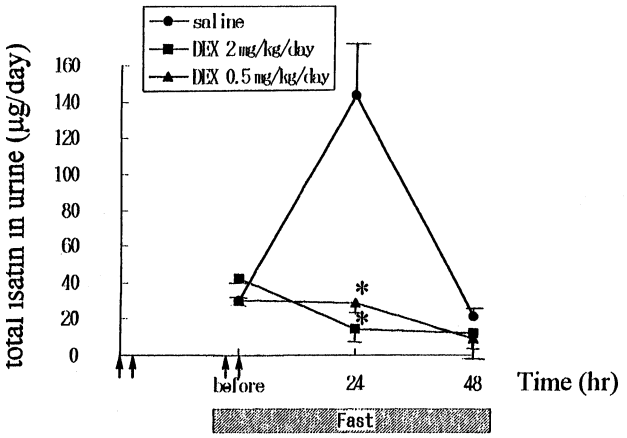

FIG. 2. Effect of DEX pretreatment at a high (2 mg/kg/day) and a low (0.5 mg/kg/day) dose on the increase in the 24-hr urinary isatin excretion during food deprivation. Each value is the mean ± SEM of 3–6 determinations in each group. Arrows indicate DEX or saline injections. DEX was administered twice daily. *$P < 0.01$, compared with saline-treated group.

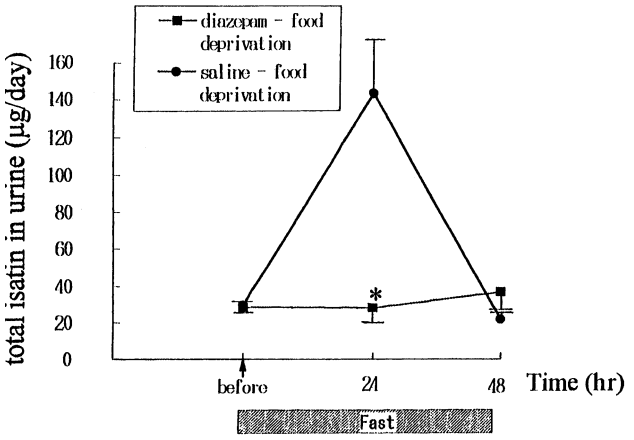

FIG 4. Effect of diazepam (1 mg/kg/day) pretreatment on the increase in the 24-hr urinary isatin excretion induced by food deprivation. Each value is the mean ± SEM of 3–6 determinations in each group. Diazepam or saline was administered i.p. after collecting urine for "before" stress at 1:00 p.m. Animals were deprived of food 30 min after injections. The arrow indicates diazepam or saline injection. *$P < 0.005$, compared with saline-treated group.

an inhibitor of dopamine-β-hydroxylase which catalyzes the last step in NA biosynthesis, failed to prevent it (data not shown).

DISCUSSION

This study was prompted by the idea that it could provide some information on the physiological role and biosynthetic pathway of isatin to determine what augments or reduces urinary isatin excretion; previous studies on isatin had focused mainly on the neurochemical or behavioral changes induced by it. We measured total isatin excretion in 24-hr urine because isatin concentrations in spot urine samples may be affected by a variety of diurnal rhythms in neuropeptides, biogenic amines, and amino acids. Isatin production, especially in states of stress, may be associated with the synthetic or degenerative pathways of the stress-related substances mentioned above. Urinary isatin excretion was augmented sharply and significantly following both types of stress (Fig. 1). These findings are the first to provide evidence that elevated urinary isatin excretion is related to stress in animals, and are consistent with earlier predictions that urinary isatin may serve as an endocoid marker of stress and anxiety.

There is general agreement that CRF acts as an integrator or regulatory factor of an organism's responses to stress. Central CRF administration effects autonomic [27], endocrine [28], and behavioral changes [29, 30] similar to those observed in animals during some kinds of stress, although other peptides, such as thyrotropin-releasing factor and somatostatin, also participate in the regulation of the autonomic nervous system [18, 31]. Some reports provide

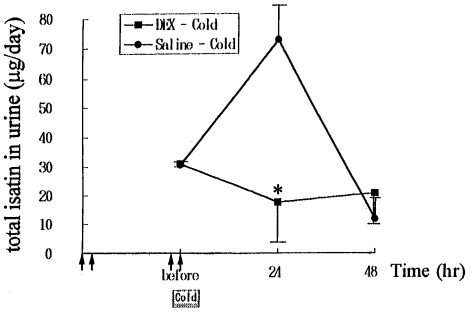

FIG 3. Effect of pretreatment with DEX (2 mg/kg/day) on the acute cold exposure-induced increase in urinary isatin excretion. Each value is the mean ± SEM of three determinations in each group. Arrows indicate DEX or saline injections administered 27 hr, 24 hr, 3 hr, and 10 min prior to cold exposure. *$P < 0.01$, compared with saline-treated group.

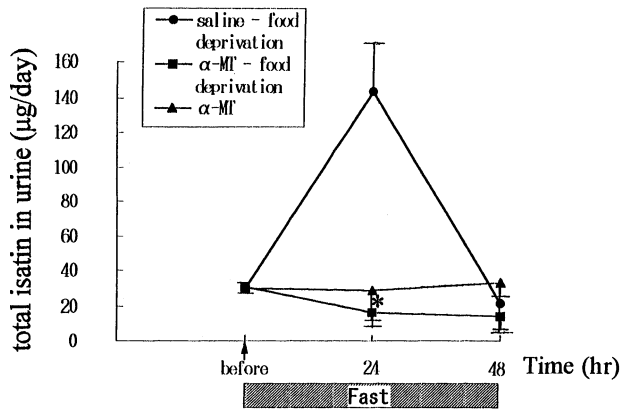

FIG 5. Effect of α-MT pretreatment (300 mg/kg) on the increase in 24-hr urinary isatin excretion during food deprivation. α-MT dissolved in saline was administered i.p. 30 min prior to the initiation of food deprivation in one group and to fed rats in another group. Saline injections accompanied drug treatments. Each value is the mean ± SEM of 4–6 determinations in each group. The arrow indicates α-MT or saline injection. *$P < 0.005$, compared with saline-treated and food-deprived group.

evidence for pronounced regional differences in the regulation of CRF mRNA by glucocorticoids and stress [24]. CRF mRNA in the PVN, which is believed to be the primary site for CRF production in response to stress-related afferent information, is negatively regulated by glucocorticoids and positively regulated by some types of stress. In this study, systemic pretreatment with the synthetic glucocorticoid DEX at a dose large enough to down-regulate the levels of CRF in the PVN markedly blocked the increase in urinary isatin excretion induced by both types of stress (Figs. 2 and 3). The preventive effect of diazepam on the increase in urinary isatin excretion (Fig. 4) may be ascribed to the antagonizing action of the drug on CRF. It is reasonable to assume that urinary isatin excretion is augmented during stress, such as acute cold exposure and food deprivation, via activation of CRF neurons.

The observation that α-MT blocked the increase of urinary isatin excretion during stress (Fig. 5) indicates that tyrosine hydroxylation, the first step in NA synthesis, could be the essential step responsible for the augmentation of isatin production. Because glucocorticoids have been demonstrated not to decrease TH activity, the mechanism responsible for the prevention of the increase in urinary isatin during stress by α-MT is different from that by DEX. TH activity increases rapidly during sympathetic stimulation [32] and during exposure to various stressors [33, 34]. Therefore, increased sympathoneural activity during stress stimulates the synthesis of DOPA in sympathetic neurons and enhances the release of newly synthesized DOPA, DA, and DOPAC into the circulation [35]. The increases in DOPA, DA, and DOPAC may be explained assuming that, under conditions of increased sympathetic activity, there is a shift in the rate-limiting step for NA synthesis from the hydroxylation of tyrosine to the hydroxylation of DA. Because noradrenergic terminals store much more NA than DA, blockade of TH may decrease rapidly the content of DA with unaltered or rather augmented responses of plasma NA and its metabolite levels, which derive from releasable NA stores in sympathetically innervated tissues [35]. α-MT markedly decreases plasma levels of DOPA and DOPAC, and markedly attenuates the enhancing effects by stress on plasma levels of DA and DA metabolites, especially DOPAC, which are dependent on sympathoneural activity. In marked contrast, plasma NA, adrenaline, and methoxyhydroxyphenylglycol levels are unaltered or rather augmented by pretreatment with α-MT during stress because releasable NA stores would not be depleted despite the blockage of NA synthesis [35]. In this experiment, a dopamine β hydroxylase inhibitor, DDC, which blocks converting DA to NA, did not reverse the increase in urinary isatin excretion produced by stress. Blockade of NA synthesis by DDC increases DA levels and DA metabolism in NA neurons, which reflect the increased neuronal activity of NA, whereas DDC causes a marked reduction of NA levels. Therefore, the results suggest that the changes in urinary isatin excretion during stress may reflect *in vivo* changes in plasma levels of DA and/or DA metabolites, which are dependent on the rate of catecholamine biosynthesis in sympathetic nerve terminals.

It is well known that both fasting and cold exposure elicit marked changes in the SNS [36–38]. There is a general tendency for fasting for more than 2 days to suppress, and for cold exposure to stimulate, the SNS in rodents despite regional heterogeneity. This can be explained by the functional importance of the SNS in thermogenesis and energy balance [38–41]. Although the short-term effect of food deprivation on the autonomic system is unclear, acute responses of urinary isatin excretion may be accounted for by the activation of vagal afferents, resulting in stimulation of an ascending adrenergic projection and leading to activation of CRF release and sympathetic outflow. Muscular exercise in food-seeking behavior may also be implicated in the increase in isatin production. Both the decreased excretion of isatin during fasting of more than 2 days and the augmented excretion following acute cold exposure (Fig. 1), then, may be attributed to changes in sympathetic activity. In a previous study [20], tribulin was detected at comparatively high levels in sympathetically innervated tissues, with the highest level being found in the superior cervical ganglion. Stress induced an increase in tribulin in kidney and heart where stress induced an increase in DA content and a decrease in NA content. These observations suggest that the elevated DA originates in the activation of the SNS rather than in MAO inhibition. The effect of diazepam on the SNS may be involved in the mechanism by which the drug exerts an inhibitory effect on the responses of urinary isatin excretion during stress (Fig. 4). In addition, the inhibitory effect of diazepam on the augmentation of urinary isatin excretion during stress may contribute to the anxiolytic action of the drug. CRF acts to stimulate sympathetic outflow. The parvocellular division of the PVN, rich in CRF cells, receives copious catecholaminergic innervation originating from the brainstem noradrenergic and adrenergic neurons. It has been suggested that CRF and locus coeruleus-noradrenergic systems participate in a positive feedback loop, with each system reinforcing the function of the other. Finally, it can be hypothesized that the increase in urinary isatin excretion in response to stress is based on the activation of both CRF neurons and noradrenergic neurons; the majority of the latter is found in the SNS. Although the intestinal flora may make a substantial contribution to isatin production [17], the basal isatin production may be accounted for by the basal levels of CRF and noradrenergic activity. Isatin may serve as an endogenously generated marker for some types of stress.

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. Chocholova L and Kolinova M, Effect of isatin (2,3-dioxoindole) on audiogenic seizures in rats and its relationship to electrographic and behavioral phenomena. *Physiol Bohemoslov* **28:** 495–502, 1979.
4. Chocholova L and Kolinova M, Effect of isatin (2,3-dioxoindole) on physiological and pathological electrographic manifestations and vigilance. *Physiol Bohemoslov* **30:** 129–137, 1981.
5. Morley JE, Farr SA and Flood JF, Isatin inhibits food intake in mice. *Eur J Pharmacol* **305:** 23–29, 1996.
6. 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.
7. Glover V, Reveley MA and Sandler M, A monoamine oxidase inhibitor in human urine. *Biochem Pharmacol* **29:** 467–470, 1980.
8. Glover V, Bhattacharya SK, Sandler M and File S, Benzodiazepines reduce stress-augmented increase in rat urine monoamine oxidase inhibitor. *Nature* **292:** 347–349, 1981.
9. Petursson H, Reveley MA, Glover V and Sandler M, Urinary MAO inhibitor in psychiatric illness. *Psychiatry Res* **5:** 335–340, 1981.
10. 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, 1995.
11. Bhattacharya SK, Mitra SK and Acharya SB, Anxiogenic activity of isatin, a putative biological factor, in rodents. *J Psychopharmacol* **5:** 202–206, 1991.
12. 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.
13. Bhattacharya SK, Clow A, Przyborowska A, Halket J, Glover V and Sandler M, Effect of aromatic amino acids, pentylenetetrazole and yohimbine on isatin and tribulin activity in rat brain. *Neurosci Lett* **132:** 44–46, 1991.
14. Doyle A and Pang FY, Urinary cortisol and endogenous monoamine oxidase inhibitor(s), but not isatin, are raised in anticipation of stress and/or arousal in normal individuals. *Stress Med* **12:** 43–49, 1996.
15. Sandler M, Clow A, Watkins PJ and Glover V, Tributin—An endocoid marker for anxiety in man. *Stress Med* **4:** 215–219, 1988.
16. Clow A, Glover V, Sandler M and Tiller J, Increased urinary tribulin output in generalised anxiety disorder. *Psychopharmacology* **95:** 378–380, 1988.
17. 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.
18. Emoto H, Koga C, Ishii H, Yokoo H, Yoshida M, and Tanaka M, A CRF antagonist attenuates stress-induced increases in NA turnover in extended brain regions in rats. *Brain Res* **627:** 171–176, 1993.
19. Eberly LB, Dudley CA and Moss RL, Iontophoretic mapping of corticotropin-releasing factor (CRF) sensitive neurons in rat forebrain. *Peptides* **4:** 837–841, 1983.
20. Armand I, Levin G and Barontini M, Stress increases endogenous benzodiazepine receptor ligand-monoamine oxidase inhibitory activity (tribulin) in rat tissues. *J Neural Transm* **71:** 29–37, 1988.
21. Maeda K, Cagampang FR, Coen CW and Tsukamura H, Involvement of the catecholaminergic input to the paraventricular nucleus and of corticotropin-releasing hormone in the fasting-induced suppression of luteinizing hormone release in female rats. *Endocrinology* **134:** 1718–1722, 1994.
22. Plotsky PM and Vale W, Hemorrhage-induced secretion of corticotropin-releasing factor-like immunoreactivity into the rat hypophyseal portal circulation and its inhibition by glucocorticoids. *Endocrinology* **114:** 164–169, 1984.
23. 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.
24. Plotsky PM, Otto S and Sapolsky RM, Inhibition of immunoreactive corticotropin-releasing factor secretion into the hypophyseal-portal circulation by delayed glucocorticoid feedback. *Endocrinology* **119:** 1126–1130, 1986.
25. 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.
26. 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, 1997.
27. Fisher LA and Brown MR, Central regulation of stress responses: Regulation of the autonomic nervous system and visceral function by corticotropin releasing factor-41. *Baillieres Clin Endocrinol Metab* **5:** 35–50, 1991.
28. Rivier CL and Plotsky PM, Mediation by corticotropin releasing factor (CRF) of adenohypophysial hormone secretion. *Annu Rev Physiol* **48:** 475–494, 1986.
29. Britton DR, Vareler M, Garcia A and Rosenthal M, Dexamethasone suppresses pituitary-adrenal but not behavioral effects of centrally administered CRF. *Life Sci* **38:** 211–216, 1986.
30. Sutton RE, Koob GF, LeMoal M, Rivier J and Vale W, Corticotropin-releasing factor produces behavioral activation in rats. *Nature* **297:** 331–333, 1982.
31. Brown MR and Fisher LA, Brain peptide regulation of adrenal epinephrine secretion. *Am J Physiol* **247:** E41–E46, 1984.
32. Weiner N, Lee FL, Dreyer E and Barnes E, The activation of tyrosine hydroxylase in noradrenergic neurons during acute nerve stimulation. *Life Sci* **22:** 1197–1216, 1978.
33. Masserano JM, Vulliet PR, Tank AW and Weiner N, The role of tyrosine hydroxylase in the regulation of catecholamine synthesis. In: Catecholamines II, Handbook of Experimental Pharmacology (Eds. Trendelenburg V and Weiner N), Vol. 90, pp. 428–469. Springer, Berlin, 1996.
34. Fluharty SJ, Snyder GL, Zigmond MJ and Stricker EM, Tyrosine hydroxylase activity and catecholamine biosynthesis in the adrenal medulla of rats during stress. *J Pharmacol Exp Ther* **233:** 32–38, 1985.
35. Kvetnansky R, Armando I, Weise VK, Holmes C, Fukuhara K, Deka-Starosta A, Kopin IJ and Goldstein DS, Plasma dopa responses during stress: Independence on sympathoneural activity and tyrosine hydroxylation. *J Pharmacol Exp Ther* **261:** 899–909, 1992.
36. Young JB and Landsberg L, Effect of concomitant fasting and cold exposure on sympathoadrenal activity in rats. *Am J Physiol* **240:** E314–E319, 1981.
37. Landsberg L and Young JB, Fasting, feeding, and regulation of the sympathetic nervous system. *N Engl J Med* **298:** 1295–1301, 1978.
38. Landsberg L and Young JB, Diet-induced changes in sympathoadrenal activity: Implications for thermogenesis. *Life Sci* **28:** 1801–1819, 1981.
39. Jansky L, Non-shivering thermogenesis and its thermoregulatory significance. *Biol Rev Camb Philos Soc* **48:** 85–132, 1973.
40. Himms-Hagen J, Cellular thermogenesis. *Annu Rev Physiol* **38:** 315–351, 1976.
41. Young JB, Saville E, Rothwell NJ and Stock MJ, Effect of diet and cold exposure on norepinephrine turnover in brown adipose tissue of the rat. *J Clin Invest* **69:** 1061–1071, 1982.