Tissue Distribution, Metabolism and Effects of Bufotenine Administered to Rats

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(Accepted 27 March 1994)

Summary—Bufotenine (*N,N*-dimethyl-5-hydroxytryptamine) is a serotonin analog reported to be hallucinogenic. Bufotenine concentrations were measured by liquid chromatography with electrochemical detection after the s.c. injection of bufotenine (1, 30 or 100 mg/kg) into rats. At 1 hr, bufotenine was high in lung, heart and blood and lower in brain and liver. No *N*-monomethyl-5-hydroxytryptamine was detected, but 5-hydroxyindoleacetic acid (5HIAA) was increased due to bufotenine metabolism. Bufotenine disappeared nearly completely by 8 hr. Bufotenine concentrations were slightly higher in hypothalamus and brain stem than in striatum or cortex; serotonin was slightly decreased, and 5HIAA was increased in these brain regions. Pargyline reduced concentrations of 5HIAA in blood and tissues after bufotenine injection; LY51641 but not deprenyl mimicked pargyline, suggesting type A not type B monoamine oxidase metabolizes bufotenine. Bufotenine injection increased serum corticosterone concentration, an effect not blocked by metergoline at a dose that blocked a similar increase elicited by quipazine. Although only 2% of the serotonin was found in platelet-poor plasma, more than 99% of the bufotenine was found in platelet-poor plasma, indicating that bufotenine is not stored in platelets. These experiments indicate that bufotenine is rapidly eliminated, in part by type A monoamine oxidase, after its injection into rats and that bufotenine penetrates the blood–brain barrier poorly.

Keywords—Bufotenine, serotonin, *N*-methylserotonin, 5-hydroxyindoleacetic acid, monoamine oxidase.

Bufotenine, *N,N*-dimethyl-5-hydroxytryptamine or *N,N*-dimethylserotonin, was named by virtue of its presence in the skin of toads (genus *Bufo*), and is said to be a hallucinogenic chemical present also in some plants (Fabing and Hawkins, 1956). On the other hand, appreciable amounts of administered bufotenine are claimed by some not to cross the blood–brain barrier, and the psychotomimetic activity of bufotenine has been questioned (see Glennon *et al.*, 1979; Lyttle, 1993). Liquid chromatographic methods with electrochemical detection useful in the analysis of serotonin and 5-hydroxyindoleacetic acid (5HIAA) are applicable to bufotenine, so we have developed a method for simultaneous assay of bufotenine, *N*-methyl-5-hydroxytryptamine (*N*-desmethylbufotenine), serotonin and 5HIAA. This method has been used to study the tissue distribution and metabolism of bufotenine in rats.

METHODS

Male Sprague–Dawley rats weighing 190–260 g were purchased from Charles River Breeding Laboratories, Portage, MI. Bufotenine monooxalate was purchased from Research Biochemicals, Inc., Natick, MA. Suspensions of bufotenine monooxalate in 5% acacia were injected s.c. at specified times before rats were decapitated. Tissues were rapidly excised, frozen on dry ice, and stored at −70°C prior to analysis.

Bufotenine, *N*-methylserotonin, serotonin and 5HIAA were assayed in tissues and in blood by liquid chromatography with electrochemical detection. The analytical system consisted of a Gilson Model 307 pump and Model 231 autosampler with a BAS LC4B amperometric detector. The column was an Alltech Econosphere 5 μ C18, 4.6 × 250 mm. The mobile phase composition was 0.1 M monochloroacetic acid, 0.5 mM ethylenediamine tetracetic acid, 1 mM 1-octanesulfonic acid sodium salt, 15% acetonitrile at a flow rate of 1.8 ml/min and temperature of 40°C. The glassy carbon electrode was set at a potential of 0.75 V for detection of all compounds. Tissues were homogenized in 10 vol of 0.1 M trichloroacetic acid. The homogenates were centrifuged at 12,000 *g* for 2 min, then 20–50 μl of the supernatant fluid was injected directly on to the chromatographic column. A 150 μl aliquot of whole blood or plasma was mixed with 500 μl distilled water in a 1.5 ml plastic centrifuge tube. After 100 μl 0.35 M $ZnSO_4$ was added and mixed, the tubes were centrifuged at 12,000 *g*

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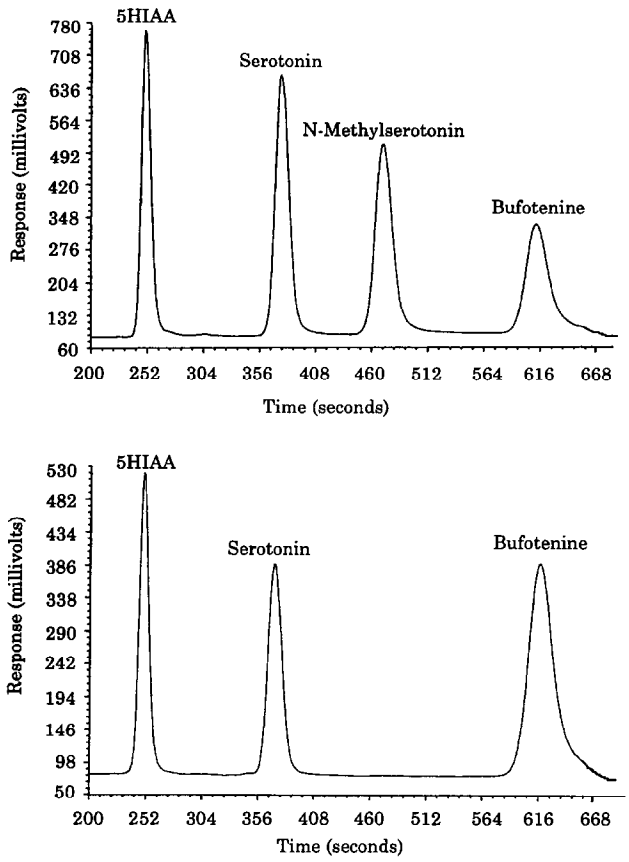

Fig. 1. LCEC detection of bufotenine, *N*-methylserotonin, serotonin and 5HIAA. Top tracing shows standards, and bottom tracing shows analysis of whole blood 1 hr after a 30 mg/kg s.c. injection of bufotenine.

for 2 min. A 350 μl aliquot of the supernatant fluid was transferred to another centrifuge tube, and 50 μl 0.4 N NaOH was added. The tubes were mixed and centrifuged at 12,000 *g* for 2 min, then 20–50 μl of the supernatant fluid was injected on to the chromatographic column. Figure 1(a) shows LCEC tracings for reference standards: butotenine, *N*-methylserotonin, serotonin and 5HIAA; Fig. 1(b) shows that in an extract of whole blood from a rat treated with bufotenine, only bufotenine, serotonin and 5HIAA (no *N*-methylserotonin) were detected. The limit of detection for bufotenine was approx 0.1 nmol/g in tissues or 0.1 nmol/ml for blood. The variability of standards extracted from tissue homogenates was 3% for brain tissue and 6% for other tissues. Serum corticosterone concentration was determined spectrofluorometrically by the method of Solem and Brinck-Johnsen (1965).

RESULTS

Table 1 shows the concentrations of bufotenine and 5HIAA in lung, heart, brain and liver 1 hr after the s.c. injection of bufotenine at doses of 1, 30 or 100 mg/kg in rats. Concentrations were highest in lung, followed by heart. Brain concentrations were very low relative to those tissues, and liver concentrations were even lower. The concentrations of 5HIAA were also markedly increased in lung and heart; they were increased in liver to a lesser degree. Brain contained highest concentrations of endogenous 5HIAA, and those concentrations were increased less by bufotenine injection than in the other

Table 1. Dose-dependence of tissue concentrations of bufotenine and 5-hydroxyindoleacetic acid (5HIAA) 1 hr after the s.c. injection of bufotenine into rats

Tissue	Dose mg/kg	Bufotenine nmol/g	5HIAA nmol/g
Lung	0	—	0.84 ± 0.07
	1	1.02 ± 0.08	1.83 ± 0.23
	30	25.27 ± 3.72	17.37 ± 2.64
	100	81.79 ± 24.53	37.26 ± 10.38
Heart	0	—	0.11 ± 0.02
	1	0.36 ± 0.01	0.24 ± 0.03
	30	12.16 ± 1.06	8.38 ± 1.14
	100	42.58 ± 5.54	23.67 ± 2.58
Brain	0	—	2.28 ± 0.06
	1	0.00 ± 0.00	2.46 ± 0.09
	30	0.88 ± 0.08	4.04 ± 0.09
	100	2.84 ± 0.40	6.68 ± 0.49
Liver	0	—	0.10 ± 0.02
	1	0.00 ± 0.00	0.16 ± 0.04
	30	0.60 ± 0.09	2.04 ± 0.28
	100	1.91 ± 0.13	4.64 ± 0.49

Bufotenine oxalate (1, 30 or 100 mg/kg s.c.) was injected 1 hr before measurements were made. Mean values ± SE for 5 rats per group are shown.

Table 2. Concentrations of bufotenine and 5-hydroxyindoleacetic acid (5HIAA) after the s.c. injection of bufotenine into rats

Tissue	Time	Bufotenine nmol/g	5HIAA nmol/g
Lung	0 time	—	0.27 ± 0.03
	1 hr	30.72 ± 2.07	18.23 ± 2.24
	2 hr	15.76 ± 1.31	15.54 ± 1.16
	4 hr	2.43 ± 0.24	2.56 ± 0.21
	8 hr	0.45 ± 0.13	0.42 ± 0.03
Blood	0 time	—	0.13 ± 0.01
	1 hr	19.83 ± 5.01	9.94 ± 1.37
	2 hr	8.70 ± 1.08	14.96 ± 1.36
	4 hr	0.73 ± 0.17	1.99 ± 0.40
	8 hr	0.03 ± 0.03	0.24 ± 0.03
Heart	0 time	—	0.09 ± 0.01
	1 hr	19.40 ± 2.45	16.63 ± 1.77
	2 hr	10.33 ± 1.01	15.54 ± 2.58
	4 hr	0.93 ± 0.16	0.92 ± 0.12
	8 hr	0.29 ± 0.03	0.11 ± 0.01
Brain	0 time	—	2.06 ± 0.06
	1 hr	1.29 ± 0.13	4.74 ± 0.17
	2 hr	0.76 ± 0.03	5.01 ± 0.14
	4 hr	0.14 ± 0.04	3.22 ± 0.15
	8 hr	0.01 ± 0.01	2.34 ± 0.08
Liver	0 time	—	0.00 ± 0.01
	1 hr	0.84 ± 0.03	2.46 ± 0.24
	2 hr	0.94 ± 0.06	2.88 ± 0.31
	4 hr	0.28 ± 0.04	0.48 ± 0.12
	8 hr	0.02 ± 0.02	0.00 ± 0.00

Bufotenine oxalate (30 mg/kg s.c.) was injected at zero time. Mean values ± SE for 5 rats per group are shown.

tissues. The ratio of 5HIAA:bufotenine concentration was highest in liver among these four tissues. In all tissues, the concentrations of bufotenine and 5HIAA were proportional to dose. No *N*-methylserotonin was detected in any tissue after any dose of bufotenine.

Table 2 shows the time course of bufotenine and 5HIAA concentrations in lung, blood, heart, brain and liver after the s.c. injection of a 30 mg/kg dose of bufotenine. Concentrations of bufotenine were highest at 1 hr in all tissues and were lower at 2 hr in all tissues except liver. Within 4 hr most of the bufotenine had disappeared, and by 8 hr bufotenine was barely detectable in lung and heart and undetectable in blood, brain and liver. The concentration of 5HIAA at 2 hr was higher than at 1 hr in blood and about the same as at 1 hr in the tissues. By 8 hr, the 5HIAA concentrations had returned essentially to control values in all tissues. No *N*-methylserotonin was found in any tissue at any of the times measured.

Rats treated with bufotenine monooxalate at 30 mg/kg s.c. were slightly stimulated for about 5–10 min after injection and showed some head searching. At 15–20 min, rats remained in the center of the cage in a flat posture and exhibited some ptosis, leg weakness, vasodilation, occasional head searching and sniffing. At 30–40 min, rats continued to remain in the center of the cage and appeared slightly depressed with some leg weakness, slow low gait, ptosis and vasodilation. At 60 min, rats were slightly depressed and showed some vasodilation and piloerection with little ptosis remaining.

The distribution of bufotenine among four different brain regions at 1 hr after the s.c. injection of bufotenine (30 mg/kg s.c.) is shown in Fig. 2. The order of decreasing concentrations of bufotenine was hypothalamus > brain stem > striatum > cortex, although the differences in concentrations across brain regions were relatively small. Serotonin concentration was slightly reduced in the bufotenine-treated rats in hypothalamus and brain stem, with smaller and nonsignificant changes in striatum and cortex. The concentrations of 5HIAA were increased significantly in all four brain regions in the bufotenine-treated rats.

Pargyline pretreatment strikingly reduced 5HIAA concentrations in all rat tissues 1 hr after the injection of bufotenine at 30 mg/kg s.c. (Fig. 3). In contrast, bufotenine concentrations were not significantly changed by pargyline pretreatment in lung, blood and heart. Bufotenine concentration was increased by pargyline

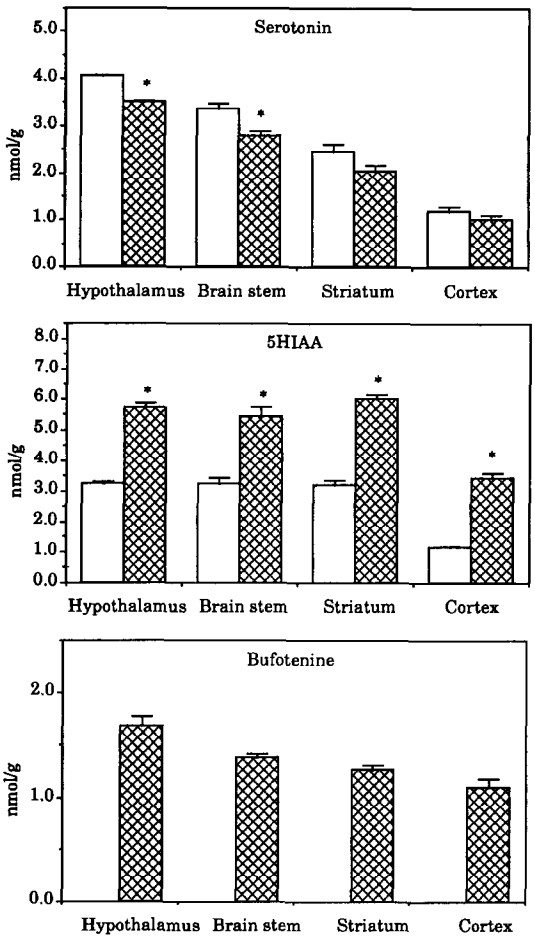

Fig. 2. 5-Hydroxyindoles in four brain regions 1 hr after the s.c. injection of bufotenine oxalate (30 mg/kg s.c.). Open bars represent control rats, cross-hatched bars represent rats treated with bufotenine. Mean values are shown for 4–5 rats per group. Asterisks indicate significant change from control value ($P < 0.05$, Tukey's test).

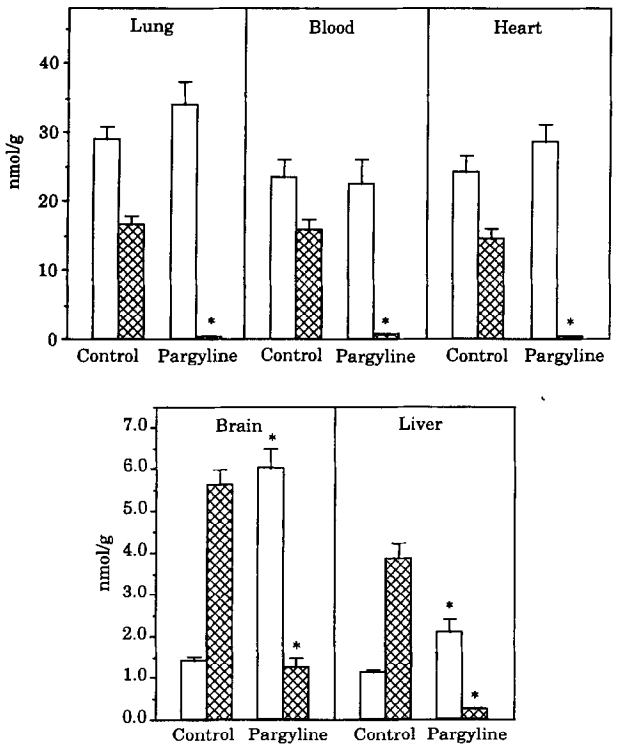

Fig. 3. Effect of pargyline pretreatment on bufotenine and 5HIAA concentrations in rat tissues 1 hr after the injection of bufotenine oxalate at 30 mg/kg s.c. Open bars represent bufotenine, cross-hatched bars represent 5HIAA. Pargyline was injected at 50 mg/kg i.p. 1 hr before bufotenine. Mean values are shown for 5 rats per group. Asterisks indicate significant differences from group treated with bufotenine alone ($P < 0.05$, Tukey's test).

pretreatment in brain (4-fold), and in liver (<2-fold). LY51641 pretreatment had effects similar to those of pargyline pretreatment (Fig. 4). In blood, 5HIAA was decreased, but bufotenine was not changed. In brain, 5HIAA was decreased, and bufotenine was increased. In

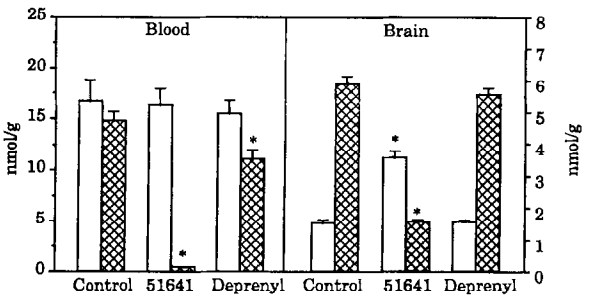

Fig. 4. Effect of LY51641 or deprenyl pretreatment on bufotenine and 5HIAA concentrations in rat blood and brain 1 hr after the injection of bufotenine oxalate at 30 mg/kg s.c. Open bars represent bufotenine, cross-hatched bars represent 5HIAA. LY51641 and deprenyl were injected at 3 mg/kg i.p. 1 hr before bufotenine. Mean values are shown for 5 rats per group. Asterisks indicate significant differences from group treated with bufotenine alone ($P < 0.05$, Tukey's test).

Table 3. Distribution of serotonin and bufotenine in rat plasma

5-Hydroxyindole measured (nmol/ml)	Platelet-rich plasma	Platelet-free plasma
Serotonin (in control rats)	4.52 ± 1.05	0.11 ± 0.01
Bufotenine (1 hr after bufotenine injection)	13.48 ± 1.03	13.42 ± 1.01

Bufotenine oxalate was injected at a dose of 30 mg/kg s.c. 1 hr before rats were killed. Mean values ± SE for 5 rats per group are shown.

contrast, pretreatment with deprenyl had no significant effect on bufotenine concentration in brain or in blood and did not affect 5HIAA concentrations in brain; deprenyl pretreatment caused only a small (but significant) decrease in 5HIAA concentration in blood (Fig. 4).

To investigate whether bufotenine was stored in blood platelets as serotonin is, we measured bufotenine in platelet-rich plasma and in platelet-free plasma at 1 hr after bufotenine injection (30 mg/kg s.c.) (Table 3). Although the concentration of serotonin in platelet-free plasma was only 2% of that in platelet-rich plasma, the concentration of bufotenine in platelet-free plasma was more than 99% that in platelet-rich plasma. Serotonin concentration in platelet-rich plasma was decreased by 71% (to 1.31 ± 0.15 nmol/ml from 4.52 ± 1.05 nmol/ml) in the rats treated with bufotenine. However, in the platelet-free plasma, serotonin concentration was increased only slightly (to 0.62 ± 0.09 nmol/ml in the bufotenine-treated rats compared to 0.11 ± 0.01 nmol/ml in controls). In a previous experiment, whole blood serotonin content was found not to be changed 1 hr after a 30 mg/kg dose of bufotenine (5.01 ± 0.77 nmol/ml in the control group, 4.96 ± 0.42 nmol/ml in the bufotenine-treated group). A likely interpretation is that bufotenine caused platelet clumping so that some platelets were spun down with the blood cell fraction, and hence were not present in the platelet-rich plasma. The 5HIAA concentrations after bufotenine injection was 25.81 ± 4.72 nmol/ml in platelet-rich plasma and 26.34 ± 4.93 nmol/ml in platelet-free plasma, indicating that 5HIAA is also not stored in platelets.

Bufotenine injection caused a dose-dependent increase in serum corticosterone concentration at 1 hr in rats [Fig. 5(a)]. Pretreatment of rats with metergoline at a 3 mg/kg i.p. dose, which prevented the quipazine-induced increase in serum corticosterone concentration, did not antagonize the increase in serum corticosterone elicited by bufotenine [Fig. 5(b)].

DISCUSSION

The present experiments show that bufotenine, like many other amine drugs, is present in high concentration in lung after it is injected into rats. Concentrations of bufotenine in brain were very low relative to those in lung and in heart after bufotenine injection, probably meaning that bufotenine does not cross the blood–brain barrier well. Serotonin is known not to cross the

blood–brain barrier, and the dimethyl substitution on the nitrogen might not be expected to have a major impact on penetration of the blood–brain barrier. Concentrations of bufotenine in liver were even lower than in brain. In all tissues studied, bufotenine disappeared relatively quickly after peak concentrations at 1 hr, suggesting it was eliminated rapidly. These findings are in general agreement with those of Sanders and Bush (1967), who gave lower doses of radioactive bufotenine i.v. to rats.

A major route of bufotenine metabolism seems to be oxidative deamination, as indicated by the large increases in 5HIAA concentration in all tissues after bufotenine injection. At 1 hr, 5HIAA concentrations in lung and heart were lower than those of bufotenine at the higher doses of bufotenine, but in brain and particularly in liver, 5HIAA concentrations were higher than those of bufotenine. The brain and liver may be able to metabolize bufotenine more rapidly than lung and heart.

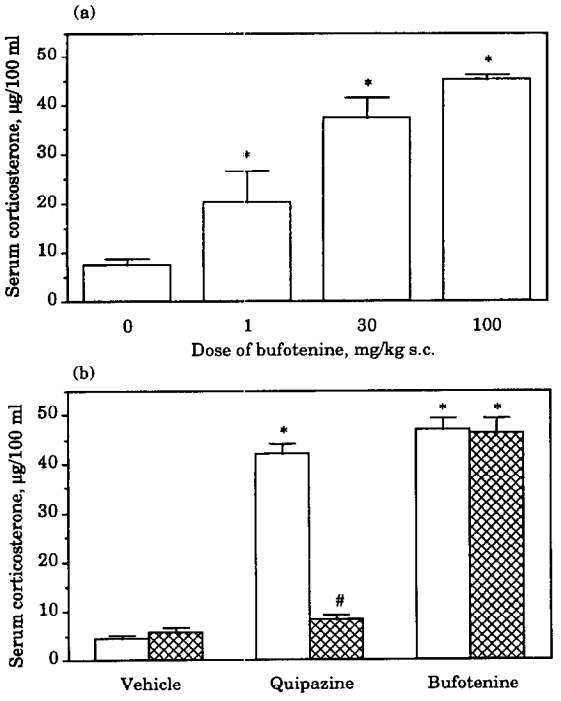

Fig. 5. Serum corticosterone increases elicited by bufotenine. (a) Dose-dependent elevation of serum corticosterone concentration 1 hr after the s.c. injection of bufotenine oxalate (1, 30 and 100 mg/kg s.c.). Mean values ± SE for 5 rats per group are shown. (b) Metergoline pretreatment antagonizes quipazine-induced but not bufotenine-induced elevation of serum corticosterone concentration in rats. Vehicle, quipazine maleate (2.5 mg/kg s.c.) or bufotenine oxalate (30 mg/kg s.c.) was injected 1 hr before rats were killed and 1 hr after metergoline (3 mg/kg i.p.). Open bars represent group without pretreatment, cross-hatched bars represent group pretreated with metergoline. Mean values ± SE for 5 rats per group are shown. Asterisks indicate significant differences from corresponding vehicle group, # indicates significant difference from corresponding open bar group.

To some extent, this may be dependent on the amount of bufotenine present, since at the lowest dose of bufotenine, 5HIAA levels in lung were higher than those of bufotenine, whereas at higher doses the opposite was true.

Bufotenine was relatively evenly distributed among the brain regions studied, the concentrations paralleling those of endogenous serotonin in the order hypothalamus > brain stem > striatum > cortex. That parallelism may suggest that bufotenine is taken up and stored in serotonin neurons to some extent. However, the distribution in blood suggests that bufotenine is not taken up and stored in blood platelets. In the control rats, only about 2% of the serotonin in platelet-rich plasma was present in the platelet-free plasma (Table 3). But in bufotenine-treated rats, over 99% of the bufotenine was present in platelet-free plasma.

The oxidative deamination of bufotenine appears to be due to the action of monoamine oxidase, as evident by the ability of pargyline to nearly completely prevent the increase in 5HIAA in all tissues that ordinarily occurred after bufotenine injection. In particular, MAO-A seems to be involved in bufotenine metabolism, as indicated by the ability of LY51641 but not deprenyl to mimic pargyline in preventing these increases in 5HIAA. LY51641 is a selective inhibitor of MAO-A, whereas deprenyl is a selective inhibitor of MAO-B at these doses (Fuller and Hemrick-Luecke, 1981). Although pretreatment with pargyline largely prevented 5HIAA formation in all tissues, such pretreatment had less dramatic effect on the concentration of bufotenine present. Only in brain and liver were bufotenine concentrations increased by pargyline pretreatment, no significant increases occurring in lung, blood or heart. These findings may indicate that bufotenine metabolism via other pathways increases after MAO-A is inhibited, or that metabolism by MAO-A is a quantitatively minor pathway.

The increase in serum corticosterone concentration produced by bufotenine is similar to the increase elicited by injection of a variety of direct-acting serotonin agonists, including non-indole compounds as well as indoles such as *N,N*-dimethyl-5-methoxytryptamine (Fuller, 1981). However, metergoline at a dose that blocked the increase in serum corticosterone elicited by quipazine did not affect the increase elicited by bufotenine. One possibility is that bufotenine's actions are not mediated by serotonin receptors and might be secondary to physiological changes (such as in body temperature or blood pressure) occurring at the high doses. Another possibility is that bufotenine increases corticosterone by acting on serotonin receptors not blocked by metergoline; e.g. metergoline has been reported not to block corticosterone increases elicited by some indirect-acting serotonin agonists and by direct agonists not acting (at least exclusively) via the same serotonin receptors that mediate the effect of quipazine (Fuller and Snoddy, 1979, 1990).

Acknowledgements—We are grateful to Susan K. Hemrick-Luecke for statistical analyses and preparation of the illustrations and to Joan Hager for typing the manuscript.

REFERENCES

Fabing H. D. and Hawkins J. R. (1956) Intravenous bufotenine injection in the human being. *Science* **123:** 886–887.

Fuller R. W. (1981) Serotonergic stimulation of pituitary-adrenocortical function in rats. *Neuroendocrinology* **32:** 118–127.

Fuller R. W. and Hemrick-Luecke S. K. (1981) Role of type A and type B MAO in the metabolism of epinephrine in rat brain. In: *Function and Regulation of Monoamine Enzymes: Basic and Clinical Aspects*. Usdin E, Weiner N and Youdim M. D. H., Eds), pp. 509–516. MacMillan Publishers, London.

Fuller R. W. and Snoddy H. D. (1979) The effects of metergoline and other serotonin receptor antagonists on serum corticosterone in rats. *Endocrinology* **105:** 923–928.

Fuller R. W. and Snoddy H. D. (1990) Serotonin receptor subtypes involved in the elevation of serum corticosterone concentration in rats by direct- and indirect-acting serotonin agonists. *Neuroendocrinology* **52:** 206–211.

Glennon R. A., Gessner P. K., Godse D. D. and Kline B. J. (1979) Bufotenine esters. *J. Med. Chem.* **22:** 1414–1416.

Lyttle T. (1993) Misuse and legend in the "toad licking" phenomenon. *Int. J. Addictions* **28:** 521–538.

Sanders E. and Bush M. T. (1967) Distribution, metabolism and excretion of bufotenine in the rat with preliminary studies of its *O*-methyl derivative. *J. Pharmac. Exp. Ther.* **158:** 340–352.

Solem J. H. and Brinck-Johnsen T. (1965) An evaluation of a method for determination of free corticosteroids in minute quantities of mouse plasma. *Scand. J. Clin. Invest.* **17 (Suppl. 80):** 1–14.