Biochemical Pharmacology, Vol. 27, pp. 2459–2461.

0006–2952/78/1015–2459$02.00/0

SHORT COMMUNICATIONS

Effect of deslanoside on brain and spinal cord levels of serotonin and 5-hydroxyindoleacetic acid and tryptophan hydroxylase activity*

(*Received* 16 *July* 1977; *accepted* 10 *January* 1978)

Data from several studies suggest that serotonin (5-HT) may be involved in mediating the toxic effects of digitalis drugs. Buterbaugh and Spratt[1] demonstrated that rats pretreated with *p*-chlorophenylalanine, an agent that inhibits the synthesis of 5-HT, required significantly larger doses of digitoxigenin i.v. to produce a lethal (respiratory arrest) response. Morgenroth *et al.*[2] obtained preliminary data indicating that the toxic i.v. doses of deslanoside administered to cats produces increases in brain 5-hydroxyindoleacetic acid (5-HIAA) level and tryptophan hydroxylase activity. A slight but significant reduction in brain 5-HT was also observed. Helke *et al.*[3] reported that drugs which interfere with serotonergic function, such as *p*-chlorophenylalanine and methysergide, increase the dose of deslanoside required to induce ventricular arrhythmias. In addition, drugs that antagonize serotonin on post-synaptic receptors were found to reverse digitalis-induced ventricular arrhythmias in both dogs and cats[4]. Finally, the neurotoxicity produced by intracerebroventricular administration of either peruvoside or ouabain in cats was found to be suppressed by either prior administration of *p*-chlorophenylalanine or 2-bromolysergic acid diethylamide, a serotonin receptor blocking agent. Perfusion of the lateral ventricles with either digitalis preparation caused a large release of serotonin[5].

The purpose of the present study was to re-examine the effects of digitalis on brain 5-HT and 5-HIAA levels, and on tryptophan hydroxylase activity.

Cats were anesthetized with α-chloralose (70–80 mg/kg, i.v.) and artificially ventilated with room air. The femoral artery and vein were catheterized for recording blood pressure and administering drugs respectively. Rectal temperature was maintained between 36 and 38° by warming the cat with radiant heat. Lead II of the electrocardiogram and the arterial blood pressure were recorded. The digitalis preparation, deslanoside, was administered by a continuous infusion of 2 μg/kg/min, i.v., until the onset of ventricular fibrillation. This occurred, on the average, after 214 ± 27 μg/kg or approximately 107 min. Brains and spinal cords were removed immediately after the onset of ventricular fibrillation. Brains were placed on ice and amygdala, hypothalamus, cerebellum, colliculi, medulla-pons, caudate nucleus, hippocampus and midbrain were quickly dissected free from surrounding tissue, frozen separately on dry ice, and stored at −80° until assay (up to 2 weeks). The cervical spinal cord was treated similarly. With each tissue, two portions were obtained by dividing the sample approximately in half. One portion was used for measurements of 5-HT and 5-HIAA while the other portion was used for the measurement of tryptophan hydroxylase activity. Control animals were treated similarly except that they were infused with normal saline for 110 min.

Serotonin was assayed by the procedure of Maickel *et al.*[6]. 5-Hydroxyindoleacetic acid proved difficult to measure with the procedure of Maickel *et al.* because of poor recoveries. Therefore, we used a modification of this technique employed by Wurtman and Fernstrom and communicated to us by Ms. Laurel Fisher. The modification included adding 0.13 ml/g of tissue of 0.1% L-cysteine to the homogenizing medium and substituting 0.5 M phosphate buffer for the 0.033 M $NaHCO_3$ during the extraction of 5-HIAA from *n*-heptane. All values reported are corrected for per cent recovery which ranged from 103 to 109 per cent for 5-HT and from 63 to 67 per cent for 5-HIAA. Fluroescence assays were performed using a Farrand MK-1 spectrophotometer. Tryptophan hydroxylase activity was determined by a modification of the method of Ichiyama *et al.*[7]. Tissue samples were homogenized in 0.01 M Tris acetate buffer (pH 7.9) containing 1 mM dithiothreitol. The homogenates were centrifuged for 30 min at 105,000 *g* and tryptophan hydroxylase was assayed in the supernatant solution. The incubation mixture (500 μl) contained the following at final concentration: Tris acetate buffer, pH 8.1 (0.1 M), 2 - amino - 4 - hydroxy - 6 - methyl - 5,6,7,8 - tetrahydropteridine (0.67 mM), dithiothreitol (3 mM), pyridoxyl phosphate (0.2 mM), catalase (60 μg/ml), ascorbic acid (1 mM), approximately 10 units L-aromatic amino acid decarboxylase prepared from beef brain stem[7], and 200 μl supernatant fluid (equivalent to 20–40 mg of tissue extract). The reaction was started by the addition of L-[1-^{14}C]tryptophan (10 μM) (New England Nuclear, Boston, MA, sp. act. 15 mCi/mmole). The reaction mixtures were sealed in glass test tubes by rubber stoppers from which were suspended plastic wells (Kontes) containing 100 μl hyamine hydroxide and accordion-folded filter paper. Samples were incubated for 45 min at 37° in a shaking water bath, and the reaction was stopped by the injection of 200 μl of 4 N perchloric acid. The $^{14}CO_2$ evolved was collected during the next 3 hr at 37°. The wells containing hyamine hydroxide and the filter papers were then placed directly into scintillation counting solution and counted in a liquid scintillation counter. Boiled tissue extracts or no tissue served as blanks[8]. Under these conditions, the assay is linear for up to 60 min and for 15–66 mg tissue. Enzyme activity was expressed as moles CO_2 evolved/g of tissue/45 min.

The data were analyzed by Student's *t*-test, for grouped data. The criterion used for statistical significance was $P < 0.05$.

The drugs used were: α-chloralose (Establissements Kuhlmann, Paris, France) and deslanoside (Sandoz Pharmaceuticals, Hanover, NJ).

The influence of deslanoside on the regional brain and spinal cord levels of 5-HT and 5-HIAA and on the activity of tryptophan hydroxylase of these tissues is presented in Table 1. In contrast to the preliminary findings presented by Morgenroth *et al.*[2], there was no significant effect of deslanoside on the levels of 5-HT and 5-HIAA. Similarly, the activity of tryptophan hydroxylase was unchanged with one exception. Animals

*Supported by grants from the U.S. Public Health Service (HE-13675 and NS-12566).

Table 1. Regional levels of serotonin and 5-hydroxyindoleacetic acid and activity of tryptophan hydroxylase in control and deslanoside-intoxicated cats*

Region	Serotonin†		5-Hydroxyindoleacetic acid†		Tryptophan hydroxylase activity‡	
	Control	Deslanoside	Control	Deslanoside	Control	Deslanoside
Midbrain	1.52 ± 0.28	2.24 ± 0.75	1.77 ± 0.37	1.90 ± 0.32	16.05 ± 2.74	17.40 ± 1.49
Hypothalamus	1.50 ± 0.13	1.67 ± 0.32	1.84 ± 0.30	1.64 ± 0.30	9.42 ± 0.70	8.45 ± 0.82
Colliculi	1.31 ± 0.21	1.39 ± 0.42	1.37 ± 0.27	1.51 ± 0.25	3.65 ± 0.25	4.48 ± 0.76
Amygdala	1.21 ± 0.17	1.31 ± 0.20	1.65 ± 0.39	1.58 ± 0.36	2.09 ± 0.38	1.76 ± 0.14
Caudate	0.98 ± 0.18	1.10 ± 0.25	1.35 ± 0.27	1.69 ± 0.28	4.30 ± 0.21	3.26 ± 0.17§
Medulla-Pons	0.86 ± 0.16	0.93 ± 0.27	0.81 ± 0.13	0.73 ± 0.14	7.58 ± 1.04	7.63 ± 1.02
Hippocampus	0.71 ± 0.13	0.91 ± 0.25	0.88 ± 0.20	0.86 ± 0.17	1.29 ± 0.07	1.34 ± 0.16
Spinal cord	0.47 ± 0.06	0.71 ± 0.20	0.40 ± 0.10	0.36 ± 0.08	1.74 ± 0.10	2.00 ± 0.24
Cerebellum					0.25 ± 0.05	0.15 ± 0.05

*Expressed as mean values with standard errors of six animals.
†Expressed as μg/g.
‡Expressed as 10^{-9} moles/g/45 min.
§$P < 0.05$, when value is compared to control value.

Table 2. Ratios of 5-HT/5-HIAA and 5-HT/tryptophan hydroxylase activity in brain regions and spinal cords of control and deslanoside-intoxicated cats*

Region	5-HT/5-HIAA		5-HT/tryptophan hydroxylase activity	
	Control	Deslanoside	Control	Deslanoside
Midbrain	0.93 ± 0.12	1.28 ± 0.22	0.14 ± 0.06	0.12 ± 0.03
Hypothalamus	0.87 ± 0.08	1.07 ± 0.12	0.17 ± 0.03	0.20 ± 0.03
Colliculi	1.06 ± 0.13	0.94 ± 0.18	0.37 ± 0.07	0.30 ± 0.05
Amygdala	0.91 ± 0.16	0.93 ± 0.10	0.67 ± 0.13	0.77 ± 0.12
Caudate	0.80 ± 0.10	0.70 ± 0.12	0.23 ± 0.04	0.36 ± 0.10
Medulla-pons	1.12 ± 0.17	1.34 ± 0.22	0.12 ± 0.03	0.12 ± 0.02
Hippocampus	0.93 ± 0.15	0.84 ± 0.13	0.57 ± 0.12	0.66 ± 0.15
Spinal cord	1.48 ± 0.26	2.13 ± 0.40	0.27 ± 0.04	0.36 ± 0.08

*Expressed as mean values with standard errors of six animals.

intoxicated with deslanoside exhibited a decrease in tryptophan hydroxylase activity in the caudate (Table 1). Ratios of 5-HT/5-HIAA and 5-HT/tryptophan hydroxylase activity were also determined, and the values are presented in Table 2. As can be seen, deslanoside administration had no significant effect on these ratios (Table 2).

The lack of effect of digitalis on most of the biochemical indices of central serotonergic function was surprising in view of the results of Gaitonde and Joglekar[5] and the preliminary findings of Morgenroth *et al.*[2]. It should be pointed out that the enhanced release of 5-HT from brain tissue by digitalis reported by Gaitonde and Joglekar was observed by administering the drug directly into the central nervous system (CNS). Our results were obtained by administering the drug intravenously. Although, for the most part, negative results were obtained with deslanoside in the present study, strong pharmacological evidence exists to indicate that a serotonin mechanism is involved in the lethal cardiotoxic effects of this drug[3, 4]. In pursuing this question, we have obtained evidence that the interaction of digitalis with serotonin mechanisms occurs primarily in the periphery[9, 10]. For example, we found no significant alteration in the arrhythmogenic effects of deslanoside when animals were pretreated by central administration of 5,7-dihydroxytryptamine[9]. In addition, we found that the antagonism of deslanoside-induced arrhythmias by methysergide did not occur because of a central nervous system effect of the drug[10]. Indeed, methysergide abolishes deslanoside-induced ventricular arrhythmias when administered to animals with their spinal cords transected.*

There is evidence that digitalis will inhibit monoamine oxidase[11, 12]. In our study, we saw no evidence of this effect as there was no difference between the 5-HT/5-HIAA ratio of control and of deslanoside-intoxicated cats.

In summary, lethal arrhythmogenic doses of deslanoside were given to cats and central nervous system tissue was removed and analyzed for 5-HT and 5-HIAA content and tryptophan hydroxylase activity. Deslanoside administration had no significant effect on 5-HT and 5-HIAA content, and except for the caudate, no effect on tryptophan hydroxylase activity.

*C. J. Helke and R. A. Gillis, unpublished observations.

†Recipient of a Predoctoral Fellowship awarded by the American Heart Association, Nation's Capitol Affiliate.
‡Recipient of Research Career Development Award HL-70673 from the National Heart and Lung Institute.

Department of Pharmacology, Georgetown University, Schools of Medicine and Dentistry, Washington, DC 20007, U.S.A.
CINDA J. HELKE†
PAMELA A. YUHANIAK
KENNETH J. KELLAR
RICHARD A. GILLIS‡

REFERENCES

1. G. G. Buterbaugh and J. L. Spratt, *J. Pharmac. exp. Ther.* **175**, 121 (1970).
2. V. H. Morgenroth, III, C. J. Helke, J. Dias Souza, B. L. Hamilton and R. A. Gillis, *Neurosci. Abstr.* **2**, Abstr. No. 126 (1976).
3. C. J. Helke, J. Dias Souza, B. L. Hamilton, V. H. Morgenroth, III and R. A. Gillis, *Nature, Lond.* **263**, 246 (1976).
4. C. J. Helke, R. A. Gillis, J. A. Quest and V. H. Morgenroth, III, *Fedn Proc.* **36**, 411 (1977).
5. B. B. Gaitonde and S. N. Joglekar, *Br. J. Pharmac.* **59**, 223 (1977).
6. R. P. Maickel, R. H. Cox, J. Sailant and F. P. Miller, *Int. J. Neuropharmac.* **7**, 275 (1968).
7. A. Ichiyama, S. Nakamura, Y. Nishizuka and O. Hayaishi, *J. biol. Chem.* **245**, 1699 (1970).
8. T. Deguichi, A. K. Sinha and J. D. Barchas, *J. Neurochem.* **20**, 1329 (1973).
9. R. A. Gillis, C. J. Helke, K. J. Kellar and J. A. Quest, *Biochem. Pharmac.* **27**, 849 (1978).
10. C. J. Helke, J. A Quest and R. A. Gillis, *Eur. J. Pharmac.* **47**, 443 (1978).
11. N. Popov and W. Forster, *Acta biol. med. germ.* **17**, 221 (1966).
12. A. R. Roy and M. L. Chatterjee, *Life Sci.* **9**, 395 (1970).

Biochemical Pharmacology, Vol. 27, pp. 2461–2463.

0006-2952/78/1015-2461$02.00/0

The effect of sodium carbenoxolone on lysosomal enzyme release

(*Received* 26 *October* 1977; *accepted* 28 *February* 1978)

Lysosomes contain a multitude of acid hydrolases capable of breaking down cellular macromolecules, including glycoproteins. Lysosomes exist in most animal tissues including the stomach [1], which is protected by a layer of gastric mucus consisting mainly of glycoproteins [2]. The protective role of this mucus and therefore the development of gastric lesions is partly dependent on the controlled activity of gastric lysosomes. Ferguson *et al.* [3] found that gastric ulcers in restrained rats were associated with reduced mucosal levels of cathepsin D, indicating rupture of lysosomal membranes and release of enzymic contents. The action of these enzymes on the mucus lining the stomach could be responsible for the 'underglycosylated' glycoprotein apparent in gastric lesions. Similar evidence exists for lysosomal involvement in drug induced gastric ulceration. 5-Hydroxytryptamine was found to induce gastric ulcers in rats, associated with a release of lysosomal enzymes [4], and the non-steroidal antiinflammatory drug phenylbutazone, at ulcerogenic doses, was found to accelerate the breakdown of lysosomes in isolated stomach preparations from the rat and rabbit [5]. The present study was designed to examine the effects of the anti-ulcerogenic drug carbenoxolone on the stability and enzyme release from lysosomes, in order to establish the possible sub-cellular modes of action of the drug.

MATERIALS AND METHODS

Phenolphthalein glucuronide, *p*-nitrophenyl phosphate, *p*-nitrophenyl-*N*-acetyl-β-D-glucopyranoside and Triton X-100 were obtained from the Sigma Chemical Company Ltd., London. Carbenoxolone sodium was a gift from Biorex Laboratories Ltd., London.

A lysosome fraction was isolated using the method of Symons *et al.* [6]. Male Wistar albino rats (body weight approx. 300 g) were used. Animals were killed by cervical dislocation, livers removed, weighed and placed in ice-cold 0.25 M sucrose. The liver was then chopped finely with scissors (2–3 mm cubes) and portions homogenised in 0.25 M sucrose using a Potter Elvejhëm homogeniser. All operations were carried out at 4°. This initial suspension was diluted to a final concentration of 10% w/v with 0.25 M sucrose and centrifuged at 750 g_{av} for 10 min to sediment unbroken cells, debris and nuclei. The supernatant was then centrifuged at 20,000 g_{av} for 20 min to sediment a 'large granule' fraction containing unbroken lysosomes. The pellet was then washed twice in 0.25 M sucrose and finally suspended in 0.25 M sucrose containing 0.05 M Tris–HCl buffer (pH 7.4). 2 ml of sucrose-buffer were added for every g of liver used. Portions (5 ml) of the lysosome suspension were added to 50 ml conical flasks, stoppered and shaken in a 37° water bath at 100 oscillations per min for 90 min. Various concentrations of carbenoxolone were added in small volumes of water (10–20 μl) to give a final concentration of 10^{-8}–10^{-3} M. Appropriate controls were always run. The level of free enzymes in lysosomal suspensions before incubation was measured by centrifuging a small volume (5 ml) at 20,000 g_{av} for 20 min and assaying enzyme activity in the supernatant. In a similar way the free enzyme levels present after control and experimental incubations were also measured. The total enzyme releasable from the lysosome preparation was taken as that released after 90 min incubation in the presence of 0.1% v/v Triton X-100.

Acid phosphatase (orthophosphoric monoester phosphohydrolase) was measured using the method of