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THE RELEVANCY OF CHANGES IN CEREBRAL 5-HYDROXYTRYPTAMINE TO RESERPINE-PRODUCED SEDATION

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Abstract—Reserpine-^{3}H and its metabolites are present in the rat brain during sedation and after disappearance of its pharmacological effects. Intracerebrally or intraventricularly administered reserpine-^{3}H shows triphasic efflux and a fairly rapid spread from the site of injection to the same sites in the brain as after its peripheral administration. Centrally injected reserpine (500 μg) did not lower cerebral, but did lower blood 5-hydropytryptamine (5-HT) levels 50 per cent in the first hour. The same amount given intravenously caused 50 per cent depletion of both blood and cerebral 5-HT within 1 hr. Cerebral depletion of 5-HT could not be related to the mobilization of corticosteroids nor to the inhibition of monoamine oxidase. When administered intracerebrally or intraventricularly, 500 μg reserpine was necessary to induce central effects in rats. This amount is far in excess of that found in the brain of sedated rats after peripheral injection of reserpine. Intracerebrally administered reserpine did not lead to depletion of 5-HT in the brain coincident with sedation. Neither the rate of 5-HT depletion nor the amount of reserpine present in the brain could be correlated with the state of sedation. It is concluded that the reserpine-produced central effects, *per se*, are not directly related to changes in the cerebral 5-HT content.

THE ROLE of 5-HT in reserpine-produced sedation continues to retain its controversial nature. The concept[1] that the central effects of reserpine are due to changes in free 5-HT of the brain is now seldom advocated. Some of the experimental evidence[2–4] in support of this view was not confirmed by others.[5–8] Subsequent observations convincingly demonstrated that sedation was elicited by reserpine in animals depleted of cerebral 5-HT, either by tryptophan deficiency[9–11] or by administration of *p*-chlorophenylalanine.[12] A recent study[13] suggests that the initial rate of 5-HT release is now claimed to be the cause of the pharmacological effects of reserpine.

The quantitative relation of reserpine-produced sedation to concomitant changes in cerebral 5-HT levels after direct administration of this drug to the brain has not been explored. This is somewhat surprising, since the understanding of the "direct drug action" of reserpine on 5-HT metabolism in the brain is seriously obscured by important systemic effects which are a consequence of the drug's peripheral administration. Earlier studies on the action of intraventricularly (i.vtr.) injected reserpine in cats[14–16] and dogs[17] were only tangential to its biochemical effects. Nevertheless, a significant difference in the dose–effect relationship between the i.vtr. and peripherally administered drug was already apparent.

The present study is concerned with the biochemical and pharmacological correlates of intracerebrally (i.c.) and i.vtr. injected reserpine as contrasted to those that follow its peripheral administration. It is intended to show that the observed changes in levels of cerebral 5-HT and in the rate of its release are not relevant to reserpine-produced sedation.

METHODS

Animals. Male hooded rats (250–400 g) were used. The animals were kept on a standard diet with water *ad libitum.*

Intraventricular injections were given through a stereotaxically introduced 50-μl Hamilton microsyringe in the right lateral ventricle according to DeGroot's atlas[18] (coordinates A = 3·2, H = 1·0, L = 5·0). Initial placement was verified histologically on 6 animals which were lesioned with a microelectrode placed in the needle holder. The brains of reserpine-injected animals were examined grossly and in all cases the needle tracts could be seen entering the ventricle. Intracerebral injections were given at a 1 mm depth in the right cortex, 3·5 mm from both the sagittal and coronal sutures. All animals were under light ether anesthesia. Sham control animals were treated identically, even to the insertion of the empty needle, to account for possible effects due to surgical stress. Intravenous injection of chemicals or withdrawal of blood samples was done from the jugular vein of animals under ether anesthesia. After reserpine injection, animals were classified as sedated when definite signs of sedation (rating 10–12 by Brodie *et al.*[13]) were evident.

Drugs and chemicals. Pure crystalline Serpasil phosphate was generously supplied by Dr. Albert Plummer of CIBA, Ltd. Reserpine (Calbiochem) was tritiated and purified by New England Nuclear Corp. (sp. act., 50 μc/mg). Reserpine-^{3}H (randomly labeled) was further purified by the method of Sheppard *et al.*[19] and was shown to be pure by paper chromatography.[20] DL-*p*-Chlorophenylalanine was donated by Chas. Pfizer & Co.; DL-5-hydroxytryptophan (5-HTP) was from Calbiochem; 3-^{14}C-DL-5-HTP (sp. act., 21·8 mc/m-mole) was from the Nuclear Chicago Corp.; 3-^{14}C-tryptamine (sp. act., 1·3 mc/m-mole) was from the New England Nuclear Corp.

All aqueous solutions were prepared with glass double-distilled water. Reserpine-^{3}H alkaloid was dissolved in 5% ascorbic acid and aliquots of this solution not exceeding 10 μl were injected in the brain. (Ten μl of 5% ascorbic acid had no effect on the animals or on 5-HT levels when administered i.c. Intracerebral injections of aqueous Serpasil phosphate ranged from 10 to 50 μl.) DL-*p*-Chlorophenylalanine (*p*-Cl-Phe) was injected i.p. as a fine suspension according to Jequier *et al.*[21]

Analytical methods. Animals were decapitated and the tissues were removed, weighed at 4° and processed. Tissue 5-HT was extracted according to a modification of the original method.[22] Tissues were homogenized in 0·1 HCl (2:1, v/w) and the homogenizer was washed with half the initial volume of the acid; these were pooled. The homogenates were centrifuged for 15 min (28,000 *g*). The supernatants were adjusted to pH 9·8 and 1-ml samples were added to 50-ml glass-stoppered centrifuge tubes containing 5 g NaCl, 2·5 ml borate buffer (pH 10) and 3·0 ml *n*-butanol. After 10 min of shaking and 5 min of centrifuging, the butanol layer was transferred to 15-ml glass-stoppered centrifuge tubes containing 7·5 ml borate buffer. After 5 min of shaking and 5 min of centrifuging, 1·5 to 2·0 ml butanol was accurately transferred to 50-ml glass-stoppered centrifuge tubes containing 10 ml of purified heptane and 1·2

ml of 0·1 M HCl for the *o*-phthalaldehyde method (OPT)[23] or 1·5 ml of 0·05 M phosphate buffer (pH 7·0) for the ninhydrin method.[24] (This latter was the method of choice after administration of 5-HTP, since this compound does not significantly interfere with the 5-HT analysis.) After another 10 min of shaking and 5 min of centrifuging, the heptane layer was removed by aspiration. The aqueous solution was pipetted into a stoppered 15-ml centrifuge tube containing 5 ml chloroform (spectral grade). After 30 sec of vigorous shaking, the tubes were centrifuged for 5 min and the aqueous layer was developed as indicated in the tables.

The inclusion of the chloroform extraction was necessary to remove traces of interfering reserpine from the samples of brain tissue obtained after i.c. or i.vtr. injection of reserpine. Control studies with reserpine-^{3}H or 5-HT-^{14}C or with both added to brain homogenates clearly demonstrated complete removal of reserpine with no loss of 5-HT. It is to be noted that while the chloroform extraction was effective in removing reserpine from 0·1 N HCl or 0·05 M phosphate buffer, by using the direct assay[22] about 40 per cent reserpine was retained in 3 N HCl after chloroform extraction. Consequently, this method can only be used if the final extraction is done with 0·1 N HCl and adjusted to 3 N HCl after the chloroform extraction.

The reserpine content of the brain was assayed by the method of Hess *et al.*[25] To obtain consistent results, it is absolutely necessary that the entire procedure be carried out in the absence of direct light and that all samples, including standards and blanks, should have exactly the same water content. An Aminco–Bowman spectrophotofluorometer was used with slit arrangement 5, excitation at 350 mμ and fluorescence at 500 mμ (uncorrected).

Reserpine-^{3}H and its metabolites were recovered from brain tissue by differential extraction.[26] In some experiments, after injection of reserpine-^{3}H, the total radioactivity was determined by hydrolyzing discrete areas of the brain in NCS reagent (Nuclear Chicago Corp.) followed by liquid scintillation counting. All protein samples obtained from experiments with radioactive substances were dissolved in this reagent by overnight digestion at 35°.

In some experiments with DL-5-HTP-^{14}C, the 5-HT and 5-hydroxyindoleacetic acid (5-HIAA) were separated on an Amberlite X-64 column[27] as described elsewhere.[28]

Plasma corticosteroids were determined by the fluorometric micromethod of Guillemin *et al.*[29] Samples of unstressed plasma were obtained from venous blood of rats within 1·5 min after they had undergone light ether anesthesia.*

RESULTS

To test whether the sites of the central pharmacological effects of reserpine and those of its biochemical effects, such as depletion of cerebral amines, are inseparable mechanisms, a study was undertaken concerning the distribution and rate of elimination of i.c. and i.vtr. introduced reserpine-^{3}H.

In several experiments, the brains were removed immediately after i.c. or i.vtr. administration of reserpine-^{3}H. (This process is accomplished in 2–5 min.) Analysis of these brains resulted in 80–90 per cent recovery of reserpine-^{3}H. Thus, these "zero time" samples served as a check of our technique.

* We are grateful to Dr. Seymour Levine (Stanford University, Palo Alto, Calif.) for his advice on obtaining unstressed plasma for corticosteroid determinations.

After i.c. injection of 100–500 μg reserpine-^{3}H, the radioactive material rapidly spread throughout the brain. For the whole brain, the rate of efflux of i.c. administered reserpine-^{3}H is shown in Fig. 1 and can be seen to be triphasic. A rapid efflux of the excess unbound reserpine occurs during the first hour, followed by a slower rate of elimination ($T_{1/2}$ = 4·5 hr) over the next 11 hr. The half-life for the slow third phase was 24 hr and signifies, in our opinion, the structurally bound reserpine. Forty-eight hr after the i.c. injection of reserpine-^{3}H, 1·2 μg/g of reserpine is still present in the brain.

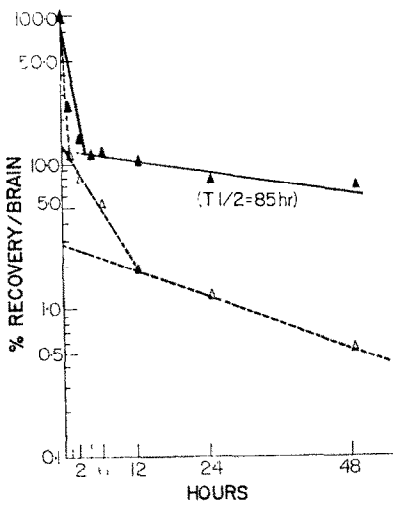

FIG. 1. Levels of total radioactivity (▲——▲) and reserpine (△- - -△) at various times in rat brain after i.c. administration of 500μg reserpine-^{3}H.

The maximum concentration of tritium for the whole brains of animals with i.p. injected reserpine (5 mg/kg; 1·3 × 10^8 dpm) was found to occur after 4 hr and totaled 0·04 per cent (Fig. 2). These i.p. recovery values are somewhat lower than those found for guinea pigs,[30] but agree closely with those found for mice.[26] The amount of reserpine-^{3}H recovered from rat brain 48 hr after its i.p. administration was 0·05 ± 0·006 μg/g. These results indicate the presence of reserpine in the brain, both during sedation and after the disappearance of its central effects. From the experiments in Fig. 2, a half-life for all radioactive material was 20 hr, while that of reserpine-^{3}H was 30 hr.

In comparison, the i.v. administration of reserpine-^{3}H (5 mg/kg; 1·3 × 10^8 dpm) causes a rapid initial rise of the drug in the brain (Fig. 3) during the first 30 min, consistent with earlier findings.[31] However, in 2 hr it attains a phase of slow elimination with a $T_{1/2}$ of 26 hr, which is not significantly different from the $T_{1/2}$ obtained from i.p. and i.c. treated animals.

The discrepancy of $T_{1/2}$ between the reserpine and total tritium in the brain after i.c. or i.v. injection (Fig. 1, 3) is due to the large amount of label reaching the brain directly. The tritium, through exchange with hydrogen of molecules of long biological half-life, may become bound into more stable structures from which it will be released

only slowly. With intraperitoneally administered randomly labeled reserpine-^{3}H, the structurally labile tritium may exchange with hydrogen in other tissue before the drug reaches the brain.

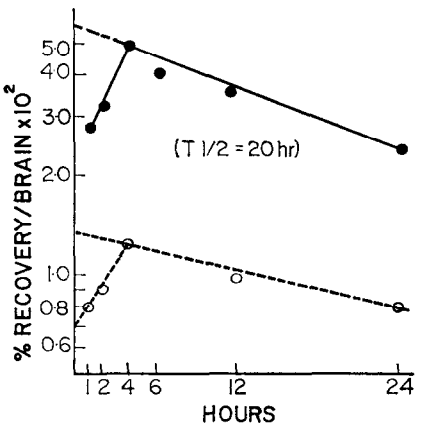

FIG. 2. Levels of total radioactivity (●——●) and reserpine-^{3}H (○ - - - ○) at various times in rat brain after i.p. administration of reserpine-^{3}H (5 mg/kg).

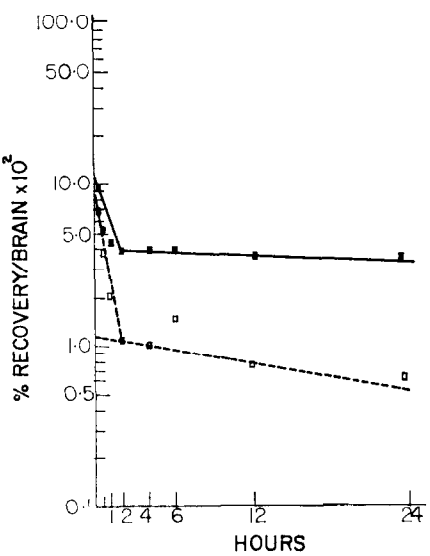

FIG. 3. Levels of total radioactivity (■——■) and reserpine-^{3}H (□ - - - □) in rat brain at various times after i.v. administration of reserpine-^{3}H (5 mg/kg).

Table 1 contains supportive data indicating that reserpine-^{3}H after i.c. administration appears at the same topographical sites in the brain as after its peripheral administration. The amount of reserpine-^{3}H present in the brain after its i.c. injection (100 μg) may be contrasted with that present after i.p. injection of the drug. Its concentration

in any area of the CNS was many times higher than that recovered from the brain after i.p. administration; yet, no sign of sedation was observed in these animals at any time. Radioautographic evidence on the cellular localization of reserpine-^{3}H after its intracerebral or peripheral administration will be presented in another report.

TABLE 1. DISTRIBUTION OF RESERPINE AND RADIOACTIVITY IN THE BRAIN 1 hr AFTER ITS ADMINISTRATION BY DIFFERENT ROUTES

	Dose of reserpine			
	0·3 mg/kg (7·3 × 10^6 dpm) i.c.		5·0 mg/kg (1·28 × 10^8 dpm) i.p.	
Area	10^5 dpm/g	Reserpine μg/g ± S.D.	10^5 dpm/g	Reserpine μg/g ± S.D.
Cerebellum	4·4	0·36 ± 0·16	0·17	0·10 ± 0·08
Medulla	3·9	0·83 ± 0·50	0·19	0·20 ± 0·10
Right cortex	11·3	6·3 ± 4·0	0·21	0·06 ± 0·05
Left cortex	1·8	0·66 ± 0·37	0·21	0·06 ± 0·05
Subcortical*	8·2	5·0 ± 2·7	0·24	0·08 ± 0·04
Central effects	None		Sedation (10–12)†	

* Includes thalamus, hypothalamus, colliculi and midbrain.
† Numerical rating as in Brodie *et al.*[13]
Each group represents 6 animals.

Further experiments were designed to test whether unilateral compensation for reserpine-produced effects could explain the absence of sedation. Serpasil phosphate injected in the brain bilaterally or subcortically in amounts totaling 100–200 μg failed to produce any sedation in any of these animals over a period of 48 hr. Only the injection of 500 μg Serpasil phosphate in the brain evoked deep sedation, within 60–90 min, which lasted for 36 hr.

Furthermore, in order to test the techniques of i.c. administration and demonstrate that drugs do reach the "sites of action" and produce the expected central effects, a number of compounds were investigated (Table 2). With all the compounds, except

TABLE 2. COMPARISON OF THE EFFECTIVE DOSE AND ONSET OF CENTRAL EFFECTS OF SEVERAL COMPOUNDS AS A FUNCTION OF THE ROUTE OF ADMINISTRATION

Compound	Mode of administration	Effective dose (mg/kg)	Time of onset central effects (min)	Ratio i.c./i.p.	Remarks
Synth. sodium fluorocitrate	i.p.	80	90	0·005	Convulsions
	i.c.	0·4	30		Convulsions
Metrazol	i.p.	60	2	0·05	Convulsions
	i.c.	3	0·5		Convulsions
Sodium nembutal	i.p.	42	15	0·07	Sedation (45–60 min)
	i.c.	3	1		Sedation (10 min)
Reserpine	i.p.	5	60	0·3	Sedation
	i.c.	1·5	60		Sedation

reserpine, not only is the time of onset of central effects considerably shortened by i.c. administration, but also their effective dose is at least an order of magnitude less than that required when they are administered peripherally.

The results in Table 3 offer further evidence for the separation of the sedative from the amine-depletive effect of reserpine. These experiments do not indicate the existence of a relationship between reserpine-produced sedation and the maximal rate constants for 5-HT depletion in rats as previously suggested.[13] For instance, the k_d for i.vtr. injected reserpine at a sedative dose is smaller by a factor of ten than that for i.p.

TABLE 3. CENTRAL EFFECTS OF RESERPINE, CEREBRAL 5-HT LEVELS AND THE RATE OF 5-HT RELEASE IN RATS*

Mode of administration	Reserpine (mg/kg)	Brain 5-HT†‡ (μg/g ± S.D.)	Rate of depletion (k_d min^{-1})§	Central effects†§
Control		0·50 ± 0·05 (10)		
i.p.	5·0	0·25 ± 0·05 (10)	0·012	Sedated 10–12
	1·5	0·45 ± 0·02 (4)	0·0015	Not sedated
i.v.	1·5	0·25 ± 0·03 (7)	0·008	Mildly sedated 7–9
	5·0	0·14 ± 0·02 (4)	0·058	Sedated 10–12
i. vtr.	1·5	0·44 ± 0·12 (7)	0·0012	Sedated 10–12
i.c.	0·3	0·45 ± 0·07 (13)	0·0019	Not sedated
	0·75	0·39 ± 0·05 (12)	0·0029	Not sedated
	1·5	0·40 ± 0·10 (9)	0·0039	Sedated 10–12
l.c. sham control		0·45 ± 0·07 (4)		

* Number of animals in parentheses.
† One hr after reserpine administration.
‡ 5-HT determined by the OPT method (see Methods).
§ Defined as in ref. 13.

reserpine, and smaller than any of the values of k_d for i.c. treated brains irrespective of sedation. Even though the k_d value reported here for i.v. treated animals is about one-half the value reported by others for rats at the same dose,[13] this value (0·012 min^{-1}) for mildly sedated animals is still three times the k_d found after i.c. reserpine at a sedative dose.

Systemically administered reserpine resulted in sedation with a concomitant drop in 5-HT in all cerebral areas in the first hour. In comparison, intracerebral administration of the drug did not produce any significant decrease in 5-HT, even though the animals became sedated with 1·5 mg/kg i.c. reserpine (Table 4). Again, the 4-hr

TABLE 4. EFFECT OF VARIOUS AMOUNTS OF RESERPINE AND THE MODE OF ITS ADMINISTRATION ON THE 5-HT CONTENT OF SELECTED AREAS OF RAT BRAIN

Condition	No. of rats	Dose (mg/kg)	(5-HT (μg/g ± S.D.)* Medulla 1 hr	Medulla 4 hr	Right cortex 1 hr	Right cortex 4 hr	Left cortex 1 hr	Left cortex 4 hr	Subcortical† 1 hr	Subcortical† 4 hr
Control	6‡		0·49 ± 0·14		0·48 ± 0·07		0·47 ± 0·13		0·65 ± 0·19	
i.p.	6	5·0	0·37 ± 0·02	0·08 ± 0·02	0·25 ± 0·06	0·12 ± 0·02	0·29 ± 0·17	0·08 ± 0·02	0·31 ± 0·13	0·11 ± 0·04
	6	1·5	0·49 ± 0·04	0·42 ± 0·08	0·38 ± 0·05	0·31 ± 0·03	0·43 ± 0·05	0·29 ± 0·02	0·49 ± 0·08	0·38 ± 0·04
i.v.	4	1·5	0·24 ± 0·10	0·11 ± 0·04	0·15 ± 0·05	0·07 ± 0·03	0·10 ± 0·05	0·07 ± 0·05	0·30 ± 0·08	0·15 ± 0·07
i.c.	6	sham	0·45 ± 0·17		0·40 ± 0·10		0·34 ± 0·04		0·58 ± 0·04	
	8	0·3	0·43 ± 0·06	0·20 ± 0·13	0·53 ± 0·09	0·27 ± 0·13	0·55 ± 0·11	0·30 ± 0·11	0·63 ± 0·17	0·36 ± 0·10
	12	0·75	0·53 ± 0·08	0·29 ± 0·12	0·36 ± 0·06	0·25 ± 0·08	0·36 ± 0·05	0·23 ± 0·08	0·40 ± 0·07	0·27 ± 0·07
	9	1·5	0·40 ± 0·16	0·18 ± 0·08	0·39 ± 0·10	0·12 ± 0·05	0·40 ± 0·11	0·17 ± 0·14	0·59 ± 0·17	0·18 ± 0·0
i.vtr.	9	1·5	0·41 ± 0·10	0·30 ± 0·12	0·31 ± 0·08	0·36 ± 0·16	0·36 ± 0·10	0·31 ± 0·12	0·54 ± 0·10	0·45 ± 0·11

* 5-HT was determined by the OPT method (see Methods).
† Includes thalamus, hypothalamus, colliculi and midbrain.
‡ Number of animals is for each data point.

values reveal no relationship between 5-HT levels and sedation, since the animals given 0·3 or 0·75 mg/kg i.c. reserpine showed significant depletion of cerebral 5-HT in all areas without any sign of sedation.

Additional experiments with 15 rats pretreated for 3 days with daily 300 mg/kg i.p. *p*-Cl-Phe showed an average 0·25 ± 0·10 μg/g of cerebral 5-HT and 0·23 ± 0·09 μg/g in 8 of them when given 500 μg i.c. reserpine. These animals, however, became deeply sedated within the first hour, thus confirming earlier reports with peripherally injected reserpine.[12]

In view of the lack of any change in cerebral 5-HT within the first 60 min, the possibility of monoamine oxidase inhibition by i.c. reserpine was investigated.

The method of Wurtman and Axelrod[32] based on oxidation of tryptamine-^{14}C was used to test the possible *in vivo* and *in vitro* inhibitory effect of reserpine on brain MAO activity. These experiments failed to show any significant difference between the cerebral MAO activity of controls and of animals receiving 500 μg i.c. reserpine after either 30 min or 1 hr. In brain homogenates from untreated rats, the MAO activity was found to be 2·8 mμmole/mg wet tissue/hr of tryptamine-^{14}C oxidized. Addition of as much as 1×10^{-3} M reserpine to the system had no effect.

In further experiments (Table 5) the effect of centrally injected reserpine on blood 5-HT levels was investigated and compared with the effect of peripherally administered

TABLE 5. CHANGES IN 5-HT AND CORTICOSTEROID LEVELS RELATIVE TO VARIOUS ROUTES OF RESERPINE ADMINISTRATION

Mode of administration	Reserpine (mg/kg)	5-HT ± S.D. (at 1 hr)*				Plasmacorticosteroids (μg/100 ml ± S.D.)		Central effects†
		Brain		Blood				
		(μg/g)	% control	(μg/ml)	% control	1 hr	4 hr	at 1 hr
Control		0·50 ± 0·05 (12)	100	1·28 ± 0·45 (4)	100	18 ± 2·9	5 ± 1·8	
Sham i.c. control						9 ± 2·6	5 ± 1·5	
i.c.	1·5	0·45 ± 0·07 (9)	90	0·66 ± 0·10 (4)	52	39 ± 6·6	32 ± 3·7	10–12
i.v.	0·5	0·46 ± 0·07 (4)	92	0·62 ± 0·14 (4)	48			none
	1·5	0·25 ± 0·05 (8)	50	0·52 ± 0·14 (4)	41			7–9
i.p.	1·5	0·45 ± 0·02 (4)	90	0·75 ± 0·12 (4)	59	15 ± 5·0	34 ± 10	none
	5·0	0·25 ± 0·02 (10)	50	0·36 ± 0·09 (4)	29	33 ± 5·6	40 ± 8·0	10–12

* 5-HT was determined by the OPT method (see Methods). Number of animals is in parentheses.
† Defined as in ref. 13.

drug. Interestingly, the i.c. reserpine (500 μg), while it did not change the cerebral 5-HT levels in 1 hr, did lower the blood 5-HT by 50 per cent. At the same time, the same amount of reserpine (500 μg) when administered i.v. caused a 50 per cent depletion of 5-HT in the brain *and* in the blood within 1 hr and a 75 per cent depletion of cerebral 5-HT in 4 hr. A 0·5 mg/kg dose of i.v. reserpine affected only the blood 5-HT level.

During the exploration of the possible systemic effects, we have concluded that cerebral depletion of 5-HT is not related to mobilization of corticosteroids, since the plasma level of corticosteroids displayed a much faster and greater increase in those animals that were given i.c. reserpine (1·5 mg/kg) than those given i.p. (1·5 mg/kg) injections. This could have resulted from the i.c. reserpine reaching the pituitary more rapidly.

Experimental evidence from measuring the reserpine-^{3}H content of the hypophysis of some rats 1 hr after i.c. injection of 500 μg reserpine-^{3}H indicates the presence of 1 per cent of the total injected radioactivity and about 0·5 to 0·8 per cent of the actual reserpine-^{3}H. On a per gram basis, this could represent an almost 30 per cent concentration of reserpine-^{3}H by the pituitary.

Reports of others[33] have demonstrated a reserpine-induced hypersecretion of ACTH after peripheral administration of the drug, which was followed by a lowering of the pituitary hormone and "an inhibition of pituitary adrenal response to stressful stimuli." This was thought to be closely related to the blockade of 5-HT storage in the brain. The results included in Table 5 confirm the increased plasma corticosterone levels, which are particularly rapid after i.c. administration of reserpine, but reveal no correlation between this and cerebral 5-HT levels. These observations, however, are not to be understood as being presented in support of a hypophyseal pathway of action by reserpine in preference to the possibility of one of hypothalamic origin. In fact, the monoamines present in the hypothalamus do not seem to be involved in the control of pituitary ACTH secretion.[34]

TABLE 6. CENTRAL EFFECTS, CEREBRAL 5-HT AND 5-HIAA LEVELS AFTER RESERPINE IN DL-5-HTP-PRETREATED RATS*

	5-HTP			Reserpine		Brain 5-HT†					5-HIAA		
Expt.	Mode of administration	Amount per kg	10^7 dpm	Mode	Dose (mg/kg)	(μg/g)	(%)	(10^3 dpm)	(%)	Sedation in 1 hr	(10^3 dpm)	(%)	5-HIAA/5-HT
A	i.p.	200 mg	2·3	i.p.	5·0	0·77	80			yes			
	i.p.	200 mg	2·3	i.c.	1·5	1·00	104			yes			
	i.p.	200 mg	2·3			0·96	100						
B	i.c.	8·6 μg	0·55	i.p.	5·0	1·27	93			yes			
	i.c.	8·6 μg	0·55	i.c.	1·5	1·27	93			yes			
	i.c.	8·6 μg	0·55			1·37	100						
C	i.p.	200 mg	6·2	i.p.	5·0			4·2	72	yes	17·3	109	4·1
	i.p.	200 mg	6·2	i.c.	1·5			6·4	117	yes	19·6	126	3·1
	i.p.	200 mg	6·2					5·8	100		15·6	100	2·7

* Each group represents 6 animals.
† 5-HT by the ninhydrin method, duplicate determinations; all values 1 hr after reserpine.

In order to test further the relevance of changes in cerebral 5-HT to reserpine-produced central effects, animals were given varying amounts of i.p. C^{14}-DL-5-HTP 1 hr prior to administration of Serpasil phosphate. The results of experiments A and C in Table 6 indicate that 1 hr after administration of i.c. reserpine there was no appreciable difference in either the cerebral 5-HT level or in the actual radioactivity recovered in the 5-HT fraction when compared to the controls. As expected, there was an appreciable increase in the amounts of cerebral 5-HT in the 5-HTP-treated animals as compared to controls given in Table 3.[13, 35] Therefore, administration of i.c. reserpine did not affect the ability of the brain to retain 5-HT at the control level, while the cerebral 5-HT level of animals given i.p. reserpine decreased by 20–27 per cent within 1 hr. There was absolutely no difference between the pharmacological effects produced by reserpine in either of these groups. In experiment C, the amount of 5-HIAA after its extraction was determined. Notably, the ratio of 5-HIAA/5-HT is very similar in i.c. injected animals and the controls. However, there is an appreciable increase in the relative cerebral 5-HIAA to cerebral 5-HT content and, consequently, in the ratio for the i.p. reserpinized rats. Finally, in order to increase the 5-HT level to

almost three times normal, C^{14}-5-HTP was injected i.c. (see experiment B) The mode of injection of reserpine in these animals had no effect either on C^{14}-5-HT efflux or on the central effects produced in either group as well as when compared to the control rats.

DISCUSSION

There is a considerable body of experimental evidence which opposes the view that the changes in cerebral 5-HT are causally related to the central effects brought about by reserpine. For instance, the depletion of 5-HT and the arrest of its cerebral synthesis in *p*-chlorophenylalanine-treated animals did not affect reserpine-produced sedation. Furthermore, the lack of inhibition of either MAO or tryptophan-5-hydroxylase by i.c. administered drug and the lack of concordance between the magnitude of the initial rate of 5-HT depletion and the presence of sedation are further proof that reserpine-produced sedation can be evoked without the mediation of a mechanism primarily involving 5-HT. Experimental evidence dissociating reserpine-produced depression of spontaneous motor activity and release of cerebral 5-HT[36] is consistent with the findings in this paper.

The evidence presented in this paper does not negate the established experimental facts that *parenterally* administered reserpine will produce sedation with concomitant depletion of cerebral amines, nor does it imply a preference for or against a catecholamine-mediated action. Finally, it does not allow us to reach any conclusions as to the mechanism of reserpine-produced sedation—a mechanism which appears to be rather complicated to be explained by a unitarian mode of action. The aim of this paper is solely to present the results in the hope that they bring forth further proof that the sedation produced by reserpine is separable from its effect on cerebral 5-HT. The fact that the i.c. or i.vtr. administered reserpine, in spite of its presence in various and discrete areas of the CNS, required time to elicit changes in the cerebral 5-HT levels may well indicate that the action of reserpine on the cerebral amines is through a target organ other than the brain. Indeed, Sheppard *et al.*[30] earlier suggested just such a mode of action for reserpine by stating that in such a target organ there may occur "a quantitative change in the output of some substance or substances involved in the normal functions of the central nervous system." We feel that the results of our earlier report[37] and of this paper are consonant with this view.

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REFERENCES

1. P. A. SHORE and J. S. OLIN, *J. Pharmac. exp. Ther.* **122**, 295 (1958).
2. B. B. BRODIE, D. F. FINGER, F. B. ORLANS, G. P. QUINN and F. SULSER, *J. Pharmac. exp. Ther.* **129**, 250 (1960).
3. S. GARATTINI and L. VALZELLI, *Science* **128**, 1278 (1958).
4. P. GREENGARD and G. P. QUINN, *Ann. N.Y. Acad. Sci.* **96**, 179 (1962).
5. R. LAVERTY, *J. Neurochem.* **10**, 151 (1963).
6. J. KNOLL and B. KNOLL, *Archs int. Pharmacodyn. Thér.* **133**, 310 (1961).
7. B. WERDINIUS, *Acta pharmac. tox.* **19**, 43 (1962).
8. A. J. INGENITO, *Archs int. Pharmacodyn. Thér.* **166**, 324 (1967).

9. E. M. GÁL, P. A. DREWES and C. A. BARRACLOUGH, *Biochem. Pharmac.* **8**, 32 (1961).
10. E. M. GÁL, P. A. DREWES and C. A. BARRACLOUGH, *Proc. 1st Int. Pharmac. Meet.* (Stockholm), p. 107. Pergamon Press, Oxford (1962).
11. D. J. BOULLIN, *Psychopharmacologia* **5**, 28 (1963).
12. B. K. KOE and A. WEISSMAN, *J. Pharmac. exp. Ther.* **154**, 499 (1966).
13. B. B. BRODIE, M. S. COMER, E. COSTA and A. DLABAC, *J. Pharmac. exp. Ther.* **152**, 340 (1966).
14. J. H. GADDUM and M. VOGT, *Br. J. Pharmac. Chemother.* **11**, 175 (1956).
15. E. R. JOHN, B. M. WENZEL and R. D. TSCHIRGI, *J. Pharmac. exp. Ther.* **123**, 192 (1958).
16. S. R. DAS GUPTA and T. J. HALEY, *Br. J. Pharmac. Chemother.* **12**, 529 (1957).
17. J. W. MCCUBBIN, Y. KANEKO and I. H. PAGE, *Circulation Res.* **8**, 849 (1960).
18. J. DE GROOT, *N. V. Noord-Hollandsche Uitgivers Maalschappil.* Amsterdam (1959).
19. H. SHEPPARD, R. A. LUCAS and W. H. TSIEN, *Archs int. Pharmacodyn. Thér.* **103**, 256 (1955).
20. B. P. KORZUN, A. F. ST. ANDRE and P. R. ULSHAFER, *J. Am. pharm. Ass. sci. edn.* **46**, 720 (1957).
21. E. JEQUIER, W. LOVENBERG and A. SJOERDSMA, *Molec. Pharmac.* **3**, 274 (1967).
22. D. F. BOGDANSKI, A. PLETSCHER, B. B. BRODIE and S. UDENFRIEND, *J. Pharmac. exp. Ther.* **117**, 82 (1956).
23. R. P. MAICKEL and F. P. MILLER, *Analyt. Chem.* **38**, 1937 (1966).
24. S. H. SNYDER, J. AXELROD and M. ZWEIG, *Biochem. Pharmac.* **14**, 831 (1965).
25. S. HESS, P. A. SHORE and B. B. BRODIE, *J. Pharmac. exp. Ther.* **118**, 84 (1956).
26. C. MAGGIOLO and R. J. HALEY, *Proc. Soc. exp. Biol. Med.* **115**, 149 (1964).
27. A. BERTLER, *Acta physiol. scand.* **51**, 75 (1961).
28. E. M. GÁL, M. MORGAN, S. K. CHATTERJEE and F. D. MARSHALL, JR. *Biochem. Pharmac.* **13**, 1639 (1964).
29. R. GUILLEMIN, G. W. CLAYTON, J. D. SMITH and H. S. LIPSCOMB, *Endocrinology* **63**, 349 (1958).
30. H. SHEPPARD, W. H. TSIEN, A. J. PLUMMER, E. A. PEETS, B. J. GILETTE and A. R. SCHULERT, *Proc. Soc. exp. Biol. Med.* **97**, 717 (1958).
31. P. A. SHORE, A. PLETSCHER, E. G. TOMICH, A. CARLSSON, R. KUNTZMAN and B. B. BRODIE, *Ann. N.Y. Acad. Sci.* **66**, 609, (1957).
32. R. J. WURTMAN and J. AXELROD, *Biochem. Pharmac.* **12**, 1439, (1963).
33. E. O. WESTERMAN, in *Drugs and Enzymes* (Eds. B. B. BRODIE and J. R. GILETTE), p. 381. Macmillan, New York (1965).
34. P. G. SMELIK, *Neuroendocrinology* **2**, 247 (1967).
35. E. M. GÁL, M. MORGAN and F. D. MARSHALL, JR., *Life Sci.* **4**, 1765 (1965).
36. R. D. MAGUS, F. W. KRAUSE and B. E. RIEDEL, *Biochem. Pharmac.* **13**, 943 (1964).
37. E. M. GÁL and S. A. MILLARD, *Life Sci.* **66**, 2505 (1967).