Indian J. of Pharmacy 28, N°7, 296, (1956)

LSD 346

PRELIMINARY INVESTIGATIONS ON THE INFLUENCE OF RESERPINE AND LYSERGIC ACID DIETHYLAMIDE (LSD) ON THE UPTAKE OF ^{131}I BY THE THYROID OF THE RAT.

Amiya B. Kar* **and R. J. Boscott**

(Department of Anatomy, The Medical School, University of Birmingham, Birmingham)

Little is known regarding the influence of *Rauvolfia serpentina* alkaloids on thyroid function in experimental animals apart from the unpublished observation mention by Gaunt *et al.*[1] According to this, slight enlargement of the thyroid was observed in rats guinea-pigs injected with reserpine. Ford *et al.*[2] found no alterations in B.M.R., or ^{131}I uptake by the thyroids in 10 hypertensive patients treated with mixed Rauvolfia alkaloids.

In recent years reserpine and preparations of total *Rauvolfia serpentina* alkaloids are finding increasing use in the treatment of psychiatric disorders. Lysergic acid diethylamide (LSD) is a potent hallucinogenic drug of considerable use in artificially inducing model psychoses in man.[3]

Reiss *et al.*[4, 5] have reported a trend of subnormal thyroid activity in male patients with acute schizophrenia as shown by tests on the uptake of radioiodine by the thyroid gland. We have attempted to study the influence of reserpine and LSD on thyroid function and the action of reserpine on any thyroidal effects of LSD in rats. Reserpine, rather than crude *Rauvolfia serpentine* alkaloids, was chosen because of the variability in composition of the crude preparations.

*On deputation from the Central Drug Institute, Lucknow, India under the Colombo Plan.

EXPERIMENTAL PROCEDURE

Animals.—Thirty-two male albino rats weighing 170-180 g. were used in this study and were divided into four equal groups. Care was taken to maintain uniform laboratory conditions for each group of animals receiving the drugs, as well as the control group.

Reserpine treatment.— Reserpine (Riker) was injected subcutaneously each day for 3 days in a dose of 2.5 mg./ kg. body weight per rat. The drug was dissolved in 0.25 ml. of a 1:1:2 mixture of ethanol, propylene glycol and water.[6] *Group 2* (Table 1) received reserpine alone. This treatment produced a marked sedative effect in the animals within 2-3 hours after the injection.

LSD treatment.—LSD (D-Lysergic acid diethylamide tartrate, "Delysid" Sandoz) was administered intraperitoneally in a

daily dosage of 0.6 mg. kg. body weight per rat in 1 ml. of normal saline for 3 days. On the third day an additional dose of this drug was given to each animal 2 hours before the injection of radio-iodine. *Group. 3* (Table 1) received LSD alone. No changes in behaviour of the animals were noted.

Reserpine + LSD treatment.—The dosage and the mode of administration of reserpine and LSD in the combined treatment group (*group 4*, Table 1) were as in groups 2 and 3. No changes in behaviour were noticed but the sedative effect of reserpine was marked as in group 2.

Controls.—Half of the control animals received the vehicle for reserpine (0.25 ml.) per animal daily by subcutaneous injection and the remainder normal saline (1 ml. per animal administered intraperitoneally) in a similar manner as in group 3. As there was no difference in the rate of radio-iodine uptake between the two control groups injected with reserpine vehicle and normal saline respectively, they will be referred to simply as 'controls' (*group 1*, Table 1) in the subsequent sections.

Radio-activity assay.—Carrier-free ^{131}I (10 μC. per rat) was injected subcutaneously on the third day into all the animals two hours after the administration of the second dose of LSD. The animals were killed 24 hours later and the thyroid glands were carefully removed with a portion of the trachea *in situ*. The glands were then put into test tubes containing 0.4 g. of KOH in 5 ml. of distilled water and were digested by heating over a steam bath for 1 hour.

The volume of the digest was finally made up to 10 ml. with distilled water and its radio-activity was measured in a liquid type Geiger-Müller counter[1] in conjunction with a decade scaler. All counts were corrected for background and dead time.

For autoradiographic and histological studies the thyroids were fixed in alcoholic Bouin's fluid and paraffin sections (5 micra thickness) were prepared. The stripping-firm technique[5] was employed for the preparation of autoradiographs which were exposed for 6 days. The autoradiographs were stained with dilute Ehrlich's haematoxylin. The sections for histological studies were stained with Ehrlich's haematoxylin followed by alcoholic eosin.

TABLE 1

The uptake of ^{131}I by the thyroid of rats treated with reserpine and LSD.

Group No.	Treatment	Percentage dose of ^{131}I (mean ± S.E.)
1	Controls	8.94 ± 0.596
2	Reserpine	8.64 ± 0.544
3	LSD	6.31 ± 0.560
4	LSD + Reserpine	8.51 ± 0.623

Analysis:

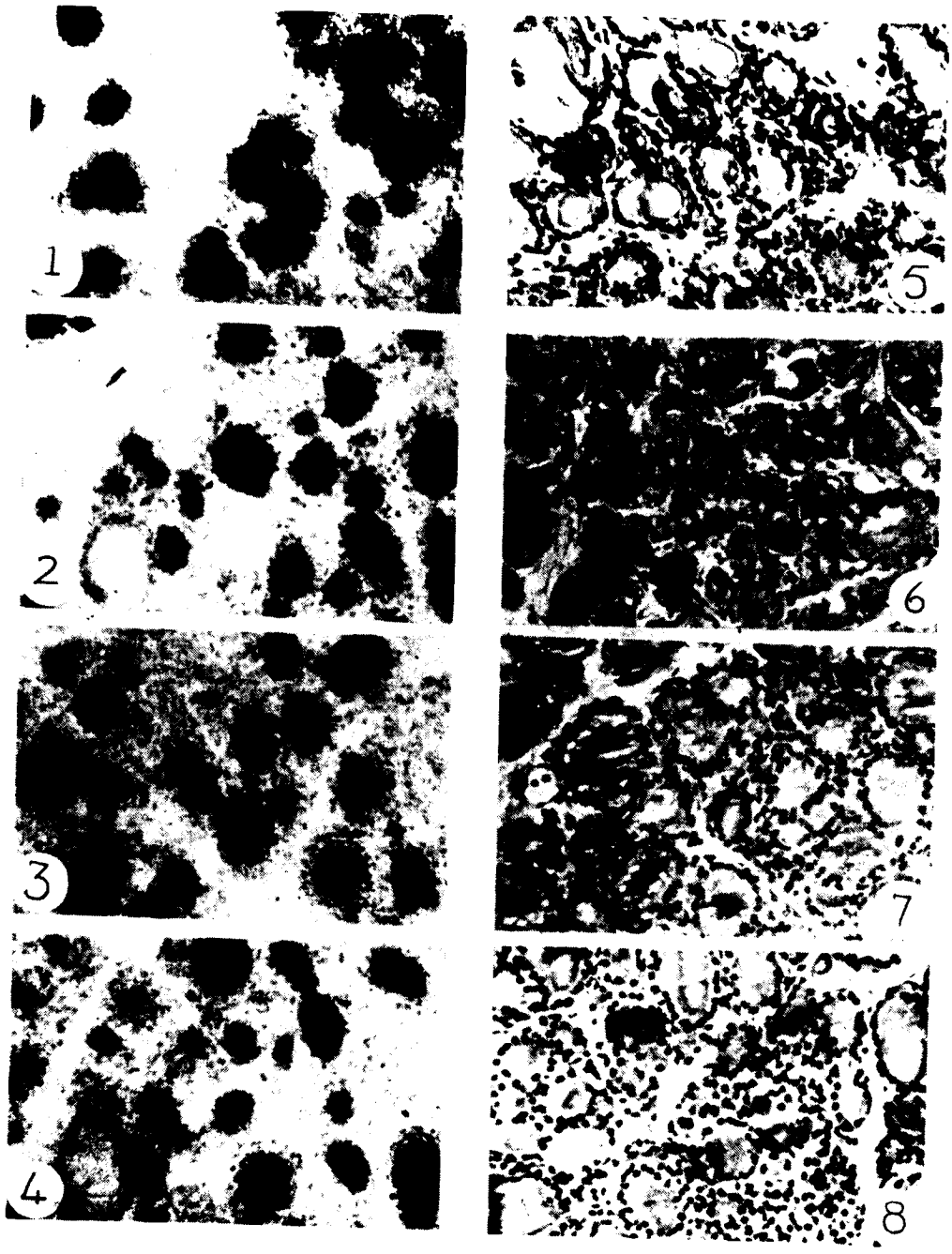
Percentage dose of ^{131}I	Group 1 vs. Group 2 —	($t = 0.372$)
	Group 1 vs. Group 3 —	($t = 3.216$ $P < 0.01 > 0.001$)
	Group 3 vs. Group 4 —	($t = 2.625$ $P = 0.02$)
	Group 1 vs. Group 4 —	($t = 0.498$)
	Group 2 vs. Group 4 —	($t = 0.157$)

RESULTS

The data on radio-iodine uptake by the thyroid are presented in Table 1.

It will be evident from Table 1 that reserpine did not influence the uptake of radio-iodine by the thyroid gland. LSD, on the other hand, significantly depressed the thyroidal uptake of ^{131}I. In rats which received LSD along with reserpine (*group 4*) the radio-iodine uptake was similar to that of the controls but was significantly greater than those treated with LSD alone. There was, however, no difference in the rats of accumulation of ^{131}I between the reserpine (*group 2*) and the LSD + reserpine groups (*group 4*).

Autoradiographic studies agreed with the above findings. Examination of autoradiographs of the thyroid of control rats showed the typical picture of distribution of radio-iodine in the follicles. The large follicles at the periphery accumulated relativity little ^{131}I as they were already distended with colloid. The



EXPLANATION OF FIGURES

(All figures are photomicrographs and are magnified × 100)

Fig. 1. Autoradiograph of the thyroid of a control rat. Note greater accumulation of radio-iodine in the central smaller follicles.

Fig. 2. Autoradiograph of the thyroid of a reserpine-treated rat. The distribution of ^{131}I is similar to that of the control.

Fig. 3. Autoradiograph of the thyroid of a LSD-treated rat. Note marked reduction in accumulation of radio-iodine in in the follicles.

small follicles, however, showed considerable accumulation of radio-iodine as they were in the process of being filled with colloid (Fig. 1). The acinar epithelium contained no radio-iodine.

The distribution of ^{131}I in the thyroid of reserpine and LSD + reserpine treated animals was more or less similar to that of the controls (Figs. 2 and 4). A marked reduction in the accumulation of radio-iodine was noted in the thyroid of LSD-treated animals. Some of the peripheral follicles were virtually devoid of ^{131}I and the central smaller ones showed considerably less accumulation of radio-iodine as compared to the other groups (Fig. 3). Radio-iodine was absent from the acinar epithlium.

The thyroids of all the groups showed normal histological structure (Figs. 5, 6, 8) except an increased vacuolation of the colloid in the LSD-treated animals (Fig. 7). No other histological change was noted in the thyroid of this *(group 3)*.

DISCUSSION

The results of the present preliminary investigations indicate that reserpine treatment alone has no direct influence on thyroid function in male rats as measured by the 24 hour uptake of a tracer dose of ^{131}I. However, reserpine did reverse the depressant effect of LSD on thyroidal uptake of radio-iodine. The results obtained with reserpine treatment alone are significant particularly in view of the clinical report of Ford *et al.*[2] who failed to observe any effect of a Rauvolfia preparation on the ^{131}I uptake by the thyroid of hypertensive patients.

Reiss *et al.*[4,5] noted hypofunction of the thyroid in male patients with acute schizophrenia. They suggested that peripheral circulatory disturbances due to the mobilisation of vasoconstrictor substances—believed to be of importance in the aetiology of schizophrenia[3]—might be responsible for the depression of thyroid function. Blood flow through the thyroid was thought to be reduced thereby causing a diminished iodine transfer to the gland, with a resultant poorer accumulation of radio-iodine.[5]

LSD is reported to block adrenergic activity by lowering the blood adrenalin after short increase during a model psychosis.[10] It is also a potent antagonist of the vasoconstrictor action of serotonin on the blood vessels of the rabbit's ear.[11] It seems unlikely, therefore, that the now observed effect of LSD on thyroid function in rats is mediated by a vasoconstrictor action similar to that suggested by Haigh *et al.*[9] for schizophrenic patients. Furthermore, the reversal of the effect of LSD by reserpine may not be due to the hypotensive action of the latter especially in view of the failure by Ford *et al.*[2] to observe any alteration in thyroid function in hypertensive patients by Rauvolfia alkaloides. However, caution must be exercised in comparing the influence of reserpine on the thyroids of normaltensive rats and hypertensive patients. Due attention should also be paid to the apparently paradoxical reports that reserpine treatment brings about disturbing mental symptoms in hypertensive patients, whereas the same drug is clearly of value in anxious and depressed ambulant patients.[12] The recent report that reserpine may possess some of the properties of serotonin which has been shown to prolong the effects of hexobarbital,[13] appears suggestive in this connection.

Hoagland *et al.*[1] have carried out extensive studies on the biochemical effects of LSD in man and experimental animals. The biochemical disturbances induced by LSD resemble closely some of the abnormalities observed in untreated schizophrenic patients. Thus, an impaired response to administered ACTH may be seen in both untreated schizophrenic patients and normals treated with LSD.

Fig. 4. Autoradiograph of the thyroid of a LSD+reserpine-treated rat. Picture of ^{131}I, distribution is similar to Figs. 1 and 2.

Fig. 5. Section through the thyroid of a control rat Normal thyroid histology.

Fig. 6. Section through the thyroid of a reserpine-treated rat. Normal thyroid histology.

Fig. 7. Section through the thyroid of a LSD-treated rat. Note vacuolation of the colloid, otherwise normal histology.

Fig. 8. Section through the thyroid of a LSD + reserpine-treated rat. Compare with Figs. 5 and 6., normal histology.

Furthermore, in both groups an impaired urinary excretion of inorganic phosphates has been observed. To what extent reserpine is able to counter these abnormalities is not known, and at present it is only possible to speculate on the mechanism by which reserpine reverses the depressive action of LSD on thyroid function.

Slingerland[15] has recently discussed the iodide concentrating mechanisms of the thyroid. According to him, it has not been possible to reduce tissue respiration without depressing iodide uptake. There are, however, several instances of inhibited iodide concentration with approximately normal respiration.[5] The cytochrome system must be complete in order to accumulate anions but blocking part of this ensyme system may not cause abnormal respiration. It is significant that trimethoxybenzioc acid moiety of reserpine is known to influence the cytochrome system,[16] but in view of the relatively low dosage of reserpine used in the present study this property may not be significant in relation to thyroid function. However, the possibility remains that reserpine may displace LSD molecules from their site of action—either the thyroid proper or the pituitary—without itself affecting the functional status of the thyroid. Whatever may be the mechanism, it is clear that further *in vitro* studies are undoubtedly required to elucidate these points and to indicate whether the now reported *in vivo* anatagonistic effects of reserpine towards LSD are mediated directly or indirectly.

Finally, it is suggested that future experiments of a nature similar to those described in this paper may prove to be of use in screening compounds of possible therapeutic value in schizophrenia, as well as in screening other hallucinogenic drugs. Studies on the effect of reserpine on the uptake of radio-iodine by the thyroid of schizophrenic patients might also lead to interesting findings.

SUMMARY

Reserpine treatment exerts no influence on the functional status of the thyroid of rats as measured by the 24 hour uptake of a tracer dose of ^{131}I. Lysergic acid diethylamide (LSD) depresses the thyroidal uptake of radio-iodine. This effect of LSD is reversed by concurrent treatment with reserpine. The possible mechanism of these actions is discussed in relation to the known influence of reserpine LSD on the endocrines and the cardiovascular system.

ACKNOWLEDGMENT

The authors wish to express their indebtedness to Professor Sir S. Zuckerman and Professor J. Elkes for their keen interest in this study and to Professor A. C. Frazer for working facilities in the Radio-Chemistry Laboratory of the Department of Pharmacology. Grateful acknowledgment is made to Dr. W. F. R. Pover for his valuable advice and help throughout the course of this investigation. Thanks are due to Dr. E. A. Ashton for statistical analysis of the data and to Miss M. Robertson for the photomicrographs. Generous contribution of reserpine by Messrs. Riker Laboratories, Nottingham, is acknowledged.

REFERENCE

1. Gaunt, R., Renzi, A., Antomchak, N., Miller, G.J., and Gilman, M., *Ann. N.Y. Acad. Sci.*, 1954, 59, 22.
2. Ford, R.V., Livesay, W.R., Miller, S.I., and Moyer, J.H., *Med. Rec. Ann.*, 1953, 47, 608.
3. Mayer-Gross, W., McAdam, W., and Walker, J.W., *J. Mental Sci.*, 1953, 99, 804.
4. Reiss, M., Haigh, C.P., Hemphill, R.E., Maggs, R., Reiss, J., and Smith, S., *J. Endocrinol.*, 1952, 8, 1.
5. Reiss, M., Hemphill, R.E., Maggs, R., Haigh, C.P., and Reiss, J., *Brit. Med. J.*, 1953, i, 906.
6. Plummer, A.J., Earl, A., Schneider, J.A., Trapold, J., and Barrett, W., *Ann. N.Y. Acad. Sci.*, 1954, 59, 8.
7. Veall, N., *Brit. J. Radiol.*, 1948, 21, 347.
8. Doniach, I., and Pele, S.R., *Brit. J. Radiol.*, 1950, 23, 472.
9. Haigh, C.P., Reiss, M., and Reiss, J., *J. Endocrinol.*, 1954, 10, 273.
10. Liddel, D.W., and Weil-Malherbe, H., *J. Neurl. Neurosurg. Psychiat.*, 1953, 16, 7.
11. Fingl, E., and Gaddum, J.H., *Fed. Proc.*, 1953, 12, 320.
12. Leading article, *Lancet*, 13, August 1955, 330.
13. Share, P.A., Silver, S.L., and Brodie, B.B., *Experientia*, 1955, 9, 272.
14. Hoagland H., Rinkel, M., and Hyde, R., *Arch. Neurol. & Psychiat.*, 1955, 73, 100.
15. Slingerland, D.W., *J. Clin. Endocrinol. & Metabol.*, 1955, 15, 131.
16. Clark, L.C., Fox, R.F., Bennington, F.C., and Morris, R., *Fed. Proc.*, 1954, 13, 27.