Biochemical Pharmacology, 1964, Vol. 13, pp. 115–127. Pergamon Press Ltd., Printed in Great Britain.

SHORT COMMUNICATIONS

Release of thyroidal serotonin by reserpine, methyldopa* and guanethidine

(*Received* 23 *August* 1963; *accepted* 17 *October* 1963)

IT WAS reported by Mayer *et al.*[1] in 1956 that reserpine, chlorpromazine, and a group of unrelated drugs inhibited the thyroid-^{131}I (iodide) trapping mechanism that incorporates inorganic iodide into organic iodinated products. Their experiments, performed on incubating calf thyroid slices in the presence of reserpine in concentrations of 8·3 μg/ml and 83 μg/ml, resulted in the conclusion that reserpine inhibited thyroid function by a *direct* action of the drug on the thyroid iodide-incorporating mechanism.

It was recently shown by Williams and Coker[2] that reserpine in a concentration of 0·5 μg/ml added to incubating rat thyroid slices stimulated the uptake of ^{131}I into the slices. These workers also showed that lysergic acid diethylamide and serotonin (0·5 μg/ml) caused a significant depression of the uptake process under the conditions of their experiments. When the effect of reserpine (in a dose of 0·15 mg/kg) on thyroidal ^{131}I uptake *in vivo* was examined, Williams and Coker found that the drug depressed ^{131}I uptake in rat thyroid glands to the extent of approximately 50% of normal. It was concluded from these experiments that the thyroid depressant action of reserpine reported previously by several workers[1, 3–7] was *indirect* and that it is caused by the transfer of peripheral serotinin into sites within the thyroid gland. This idea has long been proposed as a mechanism for the central depressant action of reserpine except that the release of serotonin from within the brain has been implicated in the depressant mechanism.[8] The conflicting data on the effect of reserpine *in vitro* on the ^{131}I uptake by thyroid slices may be due to the differences in reserpine concentration in the incubation media used by the two groups of workers, since Williams and Coker[2] were able to demonstrate an antithyroid effect of reserpine *in vivo* in the rat. It therefore seems likely that the stimulant action of the drug on ^{131}I uptake in rat thyroid slices *in vitro* reported by these workers was due to the relatively lower concentration of the drug in the medium, compared with the concentration of reserpine used by Mayer *et al.*[1] in calf slices. There is also the possibility of species difference.

The mammalian thyroid gland is fairly rich in serotonin content in some species.[9, 10] This is also true for catecholamines[11] and monoamine oxidase.[12] It might therefore be speculated that free serotonin from within the thyroid may be the mediator of the thyroid depressant action of reserpine, rather than a transfer from other peripheral sites, as suggested by Williams and Coker.[2] Experiments in this laboratory have shown that all the drugs described in the present communication depress thyrodial ^{131}I (iodide) uptake in the male rat and that this effect of reserpine and methyldopa is abolished by pretreatment of the animals with dibenzyline.[13] The most salient common property of these agents is that they release one or more biogenic amines from various tissues[14] in addition to being clinically effective antihypertensive drugs.[15] This common feature prompted an examination of the effect of these agents on thyroidal serotonin stores in an attempt to find some relationship between the effect seen on thyroid function and its level of serotonin under the influence of these compounds.

Male Sprague-Dawley rats (approximately average weight, 170 g) were used. After administration of the drugs the animals were sacrificed by decapitation, followed by immediate bloodless excision of both thyroidal lobes (and parathyroids). The glands were pooled from normal animals in groups of eight pairs per experiment and in groups of four from the drug-treated animals. Since each experiment was performed in quadruplicate, each point in Fig. 1 represents 32 animals in the normal series and 16 animals in each drug-treated series. Serotonin was estimated[16] fluorometrically† after extraction from tissue with the solvent system previously described by Shore and Olin[17] for extraction of norepinephrine, except that the following modifications were made for convenience. A 3·0-ml homogenate

* α-Methyldihydroxyphenylalanine.

† Aminco-Bowman spectrophotofluorometer.

was prepared and the volume ratio of butanol to homogenate was 5:1. After 15 min of shaking and of centrifugation, 10·0 ml of the supernatant butanol was added to a mixture of heptane and 0·1 N HCl in a volume ratio of 20:3 (ml) in the final extraction step. Recoveries of serotonin from pure solution averaged 92·5% and from homogenates, 88·5%. That this solvent system will extract serotonin from tissue has previously been verified.[18] Agreement of activation and fluorescence spectra of pure serotonin and tissue extracts was taken as additional proof that serotonin was present in the extracts. Norepinephrine added to simulated extracts did not interfere in the serotonin analysis. All drugs were administered intraperitoneally. Reserpine (2 mg/kg) was given in a volume of 1 ml/kg in the solvent described by Martindale.[19] Guanethidine sulfate (5 mg of the salt/kg) was administered in a 1 ml/kg-aqueous dilution of commercially-made ampuls of Ismelin (Ciba) containing 100 mg/ml. Methyldopa prepared immediately before use was given in a volume of 2 ml/kg in a vehicle containing 10% benzyl alcohol, 20% polysorbate-80, 40% propylene glycol, and water. Solution of the substance was accomplished with gentle warming under a running tap. Injections were made immediately after solution occurred. Control animals receiving injections of vehicles alone showed no alteration in thyroidal serotonin content except for the methyldopa control group which showed a depression of 4% at the 2-hr interval only.

It can be seen from the data in Fig. 1 that all the drugs examined depressed the level of serotonin in the rat thyroid gland. The prolonged depleting effect of reserpine is typical of that reported previously

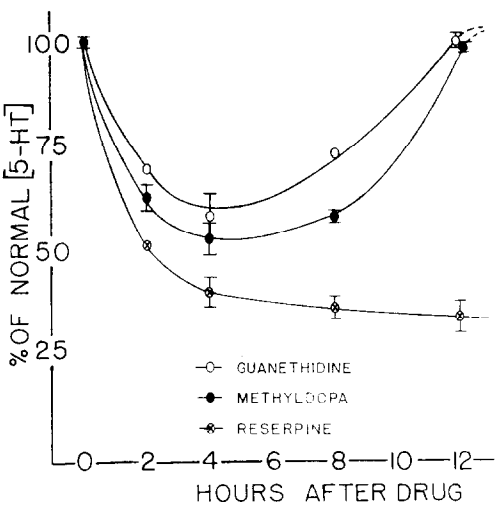

FIG. 1. The effect of a single dose of guanethidine sulfate (5 mg/kg), methyldopa (100 mg/kg), and reserpine (2 mg/kg) on thyroidal serotonin levels in the male rat. Each point in the drug-treated groups represents four experiments on 16 animals and four experiments on 32 animals in the normal series. Normal serotonin content of the rat thyroid was estimated fluorometrically to be 3·55 μg/g fresh tissue. Vertical bars are standard errors of the mean. Where no bar is seen the standard error was smaller than the diameter of the point on the graph.

in brain[20] and other tissues. Although it is not shown in the graph, the depression of thyroidal serotonin is evident even at the 60-hr interval after a single dose of reserpine. The depression of thyroidal serotonin levels by methyldopa is similar in magnitude and duration to that first reported by Smith[21] in the brain and intestine of the mouse and in the guinea pig, as reported by Hess *et al.*[22] Although the effect of guanethidine on tissue serotonin stores has not been the subject of extensive investigation, it has been stated that guanethidine lowers the concentration of serotonin in the small intestine of mice and rabbits.[23] It has been established that guanethidine releases norepinephrine from various peripheral sources.[24, 25]

It would therefore seem apparent that these substances, which are active depressants of thyroid function in the rat, decrease the level of serotonin in this gland by what is probably a release mechanism.

This has been shown for reserpine[26] and methyldopa[22] and, since guanethidine appears not to affect aromatic amino acid decarboxylase,[27, 28] the same action would presumably obtain. The common effect of these drugs on thyroidal serotonin storage offers a possible explanation for their antithyroidal action. It is not meant to imply by this that intrathyroidal serotonin release is necessary in the effect of these agents on thyroid function. The possibility of an action mediated through the hypothalamo-hypophyseal system cannot be overlooked, since reserpine can cause a variety of endocrine disturbances.[29, 30] Release of thyroidal catecholamines would present another possibility in these effects on thyroid function. Experiments aimed at offering a more concrete explanation for the antithyroid action of these agents are at present in progress.[13]

Acknowledgements—The authors wish to thank Dr. C. W. Murphy of Ciba for the gifts of reserpine and guanethidine; Dr. T. Arlie Brown of Merck for the methyldopa; and Mr. Don P. Lesiuk for providing the recovery data. This work was made possible through grants from the Medical Research Council and Warner-Lambert.

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