LSD 406
J.Pharmacol.Exper.Therap. 120,334(1957).

RELEASE OF SEROTONIN FROM BLOOD PLATELETS BY RESERPINE *IN VITRO*[1]

ARVID CARLSSON,[2] PARKHURST A. SHORE AND BERNARD B. BRODIE

Laboratory of Chemical Pharmacology, National Heart Institute, National Institutes of Health, Public Health Service, U. S. Department of Health, Education, and Welfare, Bethesda, Maryland

Received for publication February 11, 1957

Previous results have shown that reserpine releases serotonin (5-hydroxytryptamine) from various body depots, including intestine, platelets, and brain (review—Shore *et al.*, 1957). A wide variety of other centrally acting drugs have also been studied for their effects on brain serotonin. Of these, only the *Rauwolfia* alkaloids that cause sedation were found to liberate serotonin (Brodie *et al.*, 1956).

The present studies provide further information on the release of serotonin by showing that reserpine induces the discharge of serotonin from platelets *in vitro* by a mechanism that is not simple physical displacement from a cellular receptor. Comparison of the effects of a number of *Rauwolfia* drugs indicates that the ability to release serotonin from platelets *in vitro* is limited to alkaloids that exert a tranquilizing effect in animals.

METHODS AND MATERIALS. *Serotonin assay.* Serotonin in plasma was assayed by a method described by Sjoerdsma *et al.* (1957). In this procedure plasma proteins are precipitated with zinc hydroxide and the serotonin is determined directly in the acidified supernatant fluid by activation at 295 mμ and measurement of the resulting fluorescence at 550 mμ.[3]

Experimental procedures. Blood was taken from adult male rabbits, anesthetized with ether, by cannulation of the carotid artery with polyethylene tubing and collected in siliconed centrifuge tubes. One-ninth volume of one per cent disodium ethylenediamine tetraacetate was added to prevent clotting. Red cells were removed by centrifugation of the blood for 45 minutes at 150 × *g*. Five-ml. aliquots of the platelet-enriched plasma which contained 6 to 12 microgm. of serotonin per ml., were placed in siliconed 20-ml. beakers. Drugs were added by pipetting into the beakers 0.05-ml. aliquots of drug solution.[4] The samples were incubated at 37° C. in a Dubnoff metabolic incubator in an atmosphere of nitrogen. At the end of the incubation period samples of the platelet suspensions were taken for serotonin analysis. The remainder of the suspensions was transferred to siliconed centrifuge tubes and the platelets removed by centrifugation for 30 minutes at 1200 × *g*. Aliquots of the supernatant plasma were then taken for serotonin analysis.

Normally, virtually all the serotonin in plasma is localized in platelets and, in the ab-

[1] A preliminary account of this work was presented before the Society for Pharmacology and Experimental Therapeutics, Atlantic City, New Jersey, April, 1956, and the 20th International Physiological Congress, Brussels, July, 1956.

[2] Guest worker from University of Lund, Lund, Sweden.

[3] Farrand Spectrofluorometer was used in these studies.

[4] Solutions of drugs were made by dissolving various amounts of the free bases in 0.05 ml. glacial acetic acid and adding 0.5 ml. absolute ethanol, 0.5 ml. propylene glycol and 4 ml. of water. One ml. of this solution was diluted with 9 volumes of isotonic glucose solution.

We are indebted to Ciba Pharmaceutical Products, Inc., Riker Laboratories, Inc., and S. B. Penick and Co. for their liberal donations of the various *Rauwolfia* alkaloids.

sence of added drug, only a small fraction of the indole spontaneously escapes after 4 hours of incubation in the Dubnoff shaker. The release of serotonin by various drugs was therefore determined by the measurement of serotonin found in plasma after removal of platelets. Since serotonin is somewhat unstable in the presence of air, the incubations were carried out in an atmosphere of nitrogen. A number of experiments carried out in an atmosphere of air, with appropriate correction for loss of serotonin by oxidation, indicated that the rate of release was essentially the same aerobically as anaerobically.

RESULTS. *Rate of serotonin release.* One microgm. per ml. of reserpine was added to platelet suspensions and the liberation of serotonin from platelets determined after various times of incubation (fig. 1, typical of a number of experiments). Serotonin was freed slowly but continuously, and after four hours, about half the serotonin was in plasma and the rest in platelets. To establish that the release of serotonin was not due to platelet disruption, platelet counts were made in the plasma with added reserpine and found to be essentially the same as in the control plasma sample. It is of interest that the release of serotonin showed zero order kinetics; that is, a constant amount of indole passed into plasma per unit of time. The liberation of serotonin from the platelet suspensions, without added reserpine, was usually negligible, but occasionally as much as 10 per cent of the total indole appeared in plasma. When the period of incubation was longer than four hours, the spontaneous liberation of serotonin tended to increase rather sharply, presumably due to disruption of the platelets.

Serotonin release at various reserpine concentrations. Platelets were found to be extremely sensitive to the effects of reserpine and a definite release of serotonin was observed at a concentration of reserpine as low as 0.06 microgm. per ml. of plasma. With increasing concentrations of the alkaloid, the rate of serotonin liberation increased until the maximal rate was reached at a concentration of about 0.3 microgm. of reserpine per ml. of plasma. Further increase in the level of reserpine, by as much as twenty-five fold, failed to increase serotonin release beyond the limiting rate of about 40 to 50 per cent in four hours (fig. 2, typical of a number of experiments).

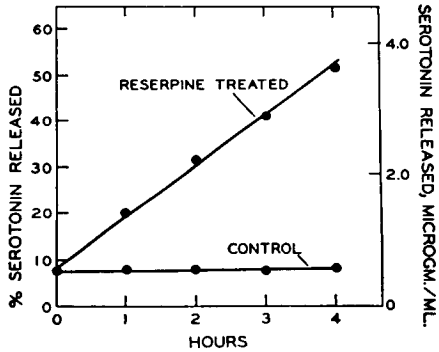

FIG. 1. Serotonin release after incubating suspension of rabbit platelets for various times with reserpine (1 microgm. per ml.).

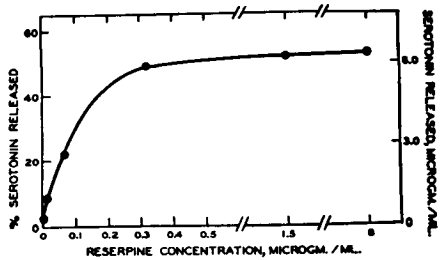

FIG. 2. Serotonin release after incubating suspensions of rabbit platelets 4 hours with various concentrations of reserpine.

TABLE 1

Release of serotonin from rabbit blood platelets in 4 hours by various drugs in vitro

Drug	Conc. of Drug	Serotonin Released
	microgm./ml.	*per cent of total**
Reserpine	1–2	47, 37, 39, 36, 47, 45
Deserpidine (Recanescine)	1–2	38, 37
Rescinnamine	1–2	37, 37
Raunescine	1–2	37, 41
11-Desmethoxyreserpine	2	39
Methyl reserpate syringate	2	47
Methyl reserpate acetoacetate	2	18
Methyl reserpate	2	8
Isoraunescine	1	6
Isoreserpine	1–2	0, 0
Reserpinine	2	0
Ajmaline	2	0
Reserpic acid	2	0
Chlorpromazine	20	5
Azacyclonol	20	2
Phenobarbital	20	0
Lysergic acid diethylamide	20	0
48/80	1	0

* Figures represent release caused solely by drug action; that is total per cent released less the small release (averaging 6 per cent) that occurred spontaneously in the control platelet suspension preparation.

The effect of various drugs on serotonin release. A number of *Rauwolfia* alkaloids were incubated with the platelet-enriched plasma preparation. The results in table 1 show that raunescine, deserpidine, rescinnamine, 11-desmethoxyreserpine and methyl reserpate syringate, all potent tranquilizing agents in the rabbit, released about 40 to 50 per cent of platelet serotonin in four hours. It cannot be concluded from this observation that these alkaloids are equally active in liberating serotonin as it is probable that the concentration of each drug was well above that required for it to exert a maximal effect. Methyl reserpate acetoacetate which exerts only a slight sedative action in rabbits dislodged only a small amount of serotonin from platelets. A number of *Rauwolfia* alkaloids that have no tranquilizing action failed to release serotonin.

Methyl reserpate and reserpic acid, hydrolytic products of reserpine, were also tested. Reserpic acid, which causes no sedative effects, released no serotonin; and methyl reserpate, which has little activity, released little or no platelet serotonin.

Several other centrally acting drugs, structurally unrelated to reserpine, were also tested: chlorpromazine and azacyclonol (Frenquel) because they have been considered to exert central effects similar to those of reserpine, LSD because it is an antagonist of reserpine and of serotonin, compound 48/80 because it has been reported to release serotonin from mast cells (Parrott and West, 1956), and phenobarbital because of its sedative action. These compounds released little or no platelet serotonin.

Effect of lowered temperature on release by reserpine. The release of serotonin from platelets was measured at room and at ice bath temperature. During a period of four hours the platelets at room temperature (25° C.) released only about 20 per cent of the serotonin, while at the temperature of the ice bath there was negligible release of the amine.

Stability of reserpine in plasma. The stability of reserpine in platelet-enriched plasma was demonstrated by incubation of the drug at a concentration of 1 microgm. per ml. for four hours. Reserpine was determined by a method which assays the parent compound, but not its hydrolytic products, methyl reserpate or reserpic acid (Hess *et al.*, 1956). The complete recovery of reserpine indicated that little if any of the alkaloid had been hydrolyzed in plasma.

Analysis of plasma before and after removal of platelets showed no significant difference in reserpine concentration. This indicates that the drug had not localized in the platelets to a significant degree.

DISCUSSION. Previous work has shown that reserpine impairs the capacity of brain and platelets *in vivo* to store serotonin (Brodie *et al.*, 1957). The present studies, which show that reserpine acts directly on platelets *in vitro* suggest that the use of platelets might be a valuable experimental procedure for study of the serotonin storage mechanism. The direct liberation of serotonin from platelets suggests that the liberation of serotonin from other tissues is also induced by a direct action of reserpine. Since reserpine releases platelet serotonin *in vitro* and *in vivo* (Shore *et al.*, 1956) at about the same rate, it is probable that the parent compound rather than a metabolic product causes release in the intact animal.

The stoichiometry of the reserpine-induced liberation of serotonin in platelets indicates that the mechanism is not simply physical displacement from a cellular receptor. Calculation of the amount of serotonin freed by 0.1 microgm. of reserpine (fig. 2) indicates that one molecule of the alkaloid effected the release of hundreds of serotonin molecules. The failure of reserpine to release serotonin at a low temperature also suggests that serotonin is released by a chemical rather than a physical action.

The mechanism of serotonin release by reserpine and the mechanism whereby tissues bind serotonin are probably different facets of the same problem. To describe serotonin as being bound is simply stating that the compound is localized and protected from monoamine oxidase which otherwise would quickly destroy it. To explain the localization, it could be assumed that the compound is anchored

onto a specific constituent of the cell. Reserpine could act by "denaturing" the storage site so that it no longer localizes serotonin. The high temperature coefficient of reserpine action and the large number of serotonin molecules liberated by a single molecule of reserpine are facts compatible with the concept that serotonin is kept within the cell by chemical forces.

The close relationship between the extent of platelet serotonin release by *Rauwolfia* alkaloids and their pharmacological activity are in accord with the thesis that their tranquilizing effects are mediated through free serotonin (Shore *et al.*, 1957). Thus, isoreserpine, a pharmacologically inactive stereoisomer of reserpine, and isoraunescine, an inactive isomer of raunescine, fail to liberate serotonin. Methyl reserpate acetoacetate causes only slight though definite sedative effects and correspondingly liberates only a small amount of serotonin. Rescinnamine, which profoundly sedates rabbits, releases a great deal of brain serotonin in this species, while in the mouse it exerts considerably less pharmacologic activity and releases much less serotonin (unpublished). Amphetamine is the only compound, not a *Rauwolfia* alkaloid, that has been reported to cause the release of brain serotonin in dogs (Paasonen and Vogt, 1956). Thus far we have been unable to induce the liberation of brain serotonin in rabbits with amphetamine even in convulsive doses.

Although chlorpromazine and reserpine induce similar central effects, the former drug does not liberate serotonin *in vivo* or *in vitro*. This tranquilizing agent, therefore, does not act through serotonin, a finding that is in accord with other evidence indicating that chlorpromazine and reserpine alter central autonomic balance by different mechanisms (Brodie and Shore, 1957).

The difference in the actions of 48/80 and reserpine are of considerable interest. The former substance has been demonstrated to rupture mast cells causing an explosive release of histamine as well as a heparin and the small amount of serotonin that they contain. While serotonin is released from mast cells (Parrott and West, 1956) and other cells by reserpine, these do not appear to be ruptured by reserpine.

SUMMARY

Reserpine incubated at 37° C. with platelet-enriched plasma induces the release of serotonin from platelets. The alkaloid does not simply displace serotonin from its binding sites for one molecule of reserpine releases hundreds of serotonin molecules. The ability of reserpine to release serotonin is temperature dependent being appreciably lower at room temperature and almost negligible at 2° C.

Only those *Rauwolfia* alkaloids with reserpine-like pharmacologic properties cause the release of serotonin from platelets. In contrast, chlorpromazine, lysergic acid diethylamide, phenobarbital and 48/80 do not release serotonin.

REFERENCES

BRODIE, B. B., SHORE, P. A., AND PLETSCHER, A.: Science, **123**: 992, 1956.

BRODIE, B. B., AND SHORE, P. A.: Annals New York Academy of Sciences, **66**: 631, 1957.

BRODIE, B. B., TOMICH, E. G., KUNTZMAN, R., AND SHORE, P. A.: THIS JOURNAL, **119**: 461, 1957.

HESS, S. M., SHORE, P. A., AND BRODIE, B. B.: THIS JOURNAL, **118:** 84, 1956.
PAASONEN, M. K., AND VOGT, M.: J. Physiol., **131:** 617, 1956.
PARROTT, J. R., AND WEST, G. B.: J. Physiol., **134:** 11P, 1956.
SHORE, P. A., PLETSCHER, A., TOMICH, E. G., KUNTZMAN, R., AND BRODIE, B. B.: THIS JOURNAL, **117:** 232, 1956.
SHORE, P. A., PLETSCHER, A., TOMICH, E. G., CARLSSON, A., KUNTZMAN, R., AND BRODIE, B. B.: Annals New York Academy of Sciences, **66:** 609, 1957.
SJOERDSMA, A., WEISSBACH, H., TERRY, L. L., AND UDENFRIEND, S.: Am. J. Med., in press, 1957.