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# EFFECT OF RESERPINE, SEROTONIN, AND 5-HYDROXYTRYPTOPHAN ON RABBIT PLATELET HISTAMINE *IN VIVO* AND *IN VITRO**

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**Abstract**—Reserpine, which releases serotonin (HT) and catecholamines from tissues, does not release histamine from various tissues of the rabbit *in vivo*, with the exception of platelets. HT and its biologic precursor, 5-hydroxytryptophan (5HTP), also effect the release of platelet histamine *in vivo*. Neither reserpine nor HT releases platelet histamine *in vitro*.

IT HAS been amply demonstrated that reserpine and other centrally active Rauwolfia alkaloids effect the release of serotonin (HT) and catecholamines from various organs of several animal species.[1,2] Administration of reserpine to rabbits also causes a decline in the histamine levels in blood[3] where most of the histamine content is localized in the platelets.[4] This finding contrasts with the observation in this laboratory that reserpine releases HT but not histamine from rabbit platelets *in vitro*.[5,6]

By taking advantage of the sensitivity of a recently developed fluorometric method for histamine assay, we have been able to show in the present studies that reserpine does not release histamine *in vivo* from various rabbit organs other than platelets. The studies further demonstrate that HT and its precursor, 5-hydroxytryptophan (5HTP), as well as reserpine, cause a decline in the histamine levels of rabbit blood *in vivo*; the results suggest that HT may be implicated in the mechanism of histamine release by reserpine *in vivo*. However, none of these substances releases histamine from rabbit platelets *in vitro*.

## METHODS

Histamine was measured by the fluorometric method of Shore *et al.*[7] Serotonin was estimated by the fluorometric method of Bogdanski *et al.*[8] after precipitation of proteins as described by Waalkes and Weissbach.[3] Estimation of the serotonin content of isolated platelets was carried out by the method described by Udenfriend *et al.*[9]

Adult male rabbits were given the various drugs by subcutaneous or intravenous injection or by slow intravenous infusion (0·2 ml/min) into an ear vein.

Studies on the release of serotonin and histamine from rabbit platelets *in vitro* were carried out as described by Carlsson *et al.*[5] In this procedure the extent of release is determined by measurement of amine levels in the supernatant fraction of plasma after removal of platelets by centrifugation.

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## RESULTS

*Effect of reserpine, HT and 5HTP on tissue histamine levels* in vivo

Analysis of brain, lung, liver, heart and kidney of rabbits given reserpine 16 hr previously failed to show a release of histamine from these tissues (Table 1). However,

TABLE 1. RABBIT TISSUE HISTAMINE LEVELS IN NORMAL RABBITS AND RABBITS GIVEN RESERPINE (2 MG/KG) 16 HR PREVIOUSLY

| Tissue | Histamine concentration (μg/g)* | |
|---|---|---|
| | Normal | Reserpine treated |
| Brain | 0·20–0·40 | 0·22–0·37 |
| Lung | 1·9 –3·1 | 1·4 –2·8 |
| Liver | 0·53–1·1 | 0·51–0·93 |
| Heart | 0·45–1·2 | 0·49–1·1 |
| Kidney | 0·48–0·90 | 0·40–0·93 |

*Each range represents from three to five experiments.

TABLE 2. EFFECT OF RESERPINE AND METHYL RESERPATE ON HISTAMINE CONTENT OF RABBIT BLOOD *in vivo*

Alkaloids were dissolved in a solvent mixture of acetic acid, ethanol, propylene glycol and water as described previously.[10] Control blood samples were taken and the drugs (2 mg/kg) were injected intravenously. Blood samples were taken 16 hr later. Values represent results of individual experiments.

| Treatment | Decrease (%) |
|---|---|
| Reserpine | 70, 67, 75 |
| Methyl reserpate | 11, 21, 15 |
| Solvent | 12, 6, 10 |

in confirmation of the findings of Waalkes and Weissbach,[3] there was a marked decline in the levels of blood histamine (Table 2). Administration of the weakly sedative Rauwolfia alkaloid, methyl reserpate, caused a decline in blood histamine levels only slightly greater than observed in rabbits treated with the alkaloid solvent alone (Table 2). Intravenous infusion or injection of HT or 5HTP caused a marked decline in the blood levels of histamine (Table 3).

Since reserpine does not cause a decrease in the concentration of circulating platelets,[10] the decline in histamine levels following reserpine administration represents a true release from these cells. HT and 5HTP administration also cause a release from platelets *in vivo*, as shown by analysis of platelets taken from these rabbits (Table 3).

*Effect of reserpine, HT and 5HTP on platelet histamine* in vitro

Addition of reserpine to platelet-rich plasma resulted in a marked release of serotonin from the platelets, as reported previously,[5] but no significant release of histamine could be detected (Table 4). Furthermore, no histamine release could be detected

TABLE 3. EFFECT OF HT AND 5HTP ON RABBIT BLOOD HISTAMINE *in vivo*

| Treatment | Decrease in Histamine Level, per cent | |
|---|---|---|
| | Whole Blood | Platelets |
| Infusion* | | |
| HT, 4 μg/kg per min | 38, 58, 48 | 18, 20 |
| HT, 8 μg/kg per min | — | 32, 28 |
| Saline | 18 | 0, 2 |
| Injection | | |
| HT† | — | 26 |
| 5HTP‡ | *27, 33*, 37, 45 | *17, 31*, 26, 38 |
| 5HTP§ | — | 52, 59 |
| Saline | 2, 9, 5 | 0, 2 |

* Infusions into an ear vein were carried out for 4 hr at the rate of 0·2 ml/min. All values represent decrease from levels in same animals before treatment.

† HT, 1 mg/kg, was injected subcutaneously every hr for 4 hr. Blood was analyzed 1 hr after the final injection.

‡ 5HTP, 50 mg/kg, was injected subcutaneously. Blood samples were taken after 2 hr (italics) and 4 hr (roman).

§ 5HTP, 100 mg/kg, was injected in two divided doses 4 hr apart. Samples were taken 22 hr after the first injection.

TABLE 4. RELEASE OF HT AND HISTAMINE FROM RABBIT PLATELETS *in vitro*

(Platelet-rich plasma was incubated with the various agents at 37 °C under nitrogen. Procedure was that described by CARLSSON *et al.*[5] Each value represents a single experiment.)

| Treatment | Amine released % | | | |
|---|---|---|---|---|
| | HT | | Histamine | |
| | 2 hr | 4 hr | 2 hr | 4 hr |
| Reserpine 1 to 3 μg/ml | 18, 20, 22 | 47, 46 | 0, 3, 0 | 6, 1 |
| HT 2 μg/ml | — | — | 0 | 2 |
| HT 40 μg/ml | — | — | 4, 2 | — |
| 5 HTP 2 μg/ml | — | — | 0, 2 | — |

following incubation of platelets with HT or 5HTP. Control studies in which histamine was added to platelet-rich plasma showed that the added histamine remained in the supernatant fraction after removal of the platelets by centrifugation.

## DISCUSSION

The observation that reserpine causes a decline in the level of histamine in rabbit blood suggests that the alkaloid might release histamine from many tissues in the same manner that it releases HT and catecholamines from their storage depots. Our findings show, however, that reserpine does not decrease the histamine levels of rabbit tissues other than blood.

A marked release of HT from tissues occurs following administration of reserpine but not methyl reserpate.[1,5] Since the same pattern is seen in the case of blood histamine release *in vivo*, it might appear that the mechanisms of release of the two amines from blood by reserpine are identical. However, while reserpine releases histamine from

platelets *in vivo*, it does not do so *in vitro* under conditions in which HT is readily released.

The restriction of the release of histamine *in vivo* to blood led us to consider that the HT released by reserpine might in turn act directly on the platelets to release histamine. There is some evidence that this series of events might occur since administration of HT or its precursor, 5HTP, caused a release of platelet histamine. Attempts to effect a release of platelet histamine *in vitro* by incubating platelets with HT or 5HTP were unsuccessful. Thus, reserpine and HT show a common pattern in their effects on platelet histamine.

At least two explanations exist which would be consistent with the difference between the results obtained *in vivo* and *in vitro*. One possible explanation is that the action of the various releasing agents *in vivo* is by a direct effect on platelets, which cannot be demonstrated *in vitro* because of the presence of the calcium-chelating agent, ethylenediaminetetra-acetate (EDTA), necessary for stabilization of isolated platelets.[11] There is some precedent for this possibility as Woolley has shown that removal of calcium ions by EDTA abolishes the uterus-contracting activity of HT *in vitro*.[12] This explanation would suggest that an interaction between calcium ions and HT is necessary for certain actions of HT, including histamine release from platelets.

Another possible explanation is that reserpine, HT and 5HTP exert a common action *in vivo* which indirectly causes a release of platelet histamine. Attempts to demonstrate this effect in a simple model system *in vitro* might then be impossible.

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