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Histamine release by reserpine from rat peritoneal mast cells *in vitro**

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WHILE reserpine is well known to lower catecholamine and serotonin levels in many tissues,[1,2] its effects on histamine stores are less consistent. *In vivo* either no change or a slight to moderate decrease in the histamine content of various tissues has been reported after the administration of large doses of drugs.[3–6] Moran and Westerholm[7] found reserpine to have no effect on the histamine concentration of rat peritoneal mast cells *in vitro*, while Mannaioni *et al.*[8] more recently reported that reserpine released histamine from neoplastic murine mast cells. In this communication we describe conditions under which reserpine does release histamine from rat peritoneal mast cells *in vitro*.

The methods used for collection and preparation of mast cells are described in detail elsewhere.[9] Peritoneal cells were removed from Sprague–Dawley rats (200–300 g; Charles River Laboratories). When required, mast cells were purified partially by briefly centrifuging and resuspending the cells in fresh medium four to eight times. The incubation medium consisted of: NaCl, 154 mM; KCl, 2·7 mM; $CaCl_2$, 0·9 mM; KH_2PO_4, 2·7 mM; Na_2HPO_4, 4·0 mM; glucose, 0·1%; human serum albumin, 0·1%; the pH was 7·0. After incubation, cells and supernatants were separated and the histamine content of both fractions was measured fluorometrically.[10] The histamine content of unincubated cells was also determined; recoveries were always greater than 95 per cent. Mast cells were stained and counted as described by Bray and Van Arsdel.[11] Reserpine phosphate (Serpasil, lyophilized) was a gift of Dr. A. J. Plummer of Ciba Pharmaceutical Co.

Table 1 illustrates that the release of histamine from mast cells depends on the cellular composition of the incubation. The per cent of histamine released increased either when the number of mast cells in the medium was reduced or when mast cells formed a larger proportion of the total cell population. The lowest concentration of reserpine that released histamine under the present conditions was 1×10^{-5} M. In one experiment (mast cells = 48,000/ml) release after 2 hr was 18 and 27 per cent as compared with 10 and 12 per cent in the presence and absence of drug respectively; in another experiment (mast cells = 95,000/ml) the equivalent values after 4 hr were 55 and 57 per cent as compared with 10 and 15 per cent.

We verified that release was due to reserpine rather than to some contaminant by taking advantage of the relative insolubility of reserpine. Saturated solutions were prepared from two different amounts of drug (different by a factor of 3). The supernatants of these contained the same concentration of reserpine but, presumably, different concentrations of a hypothetical contaminant. Release was identical with both solutions.

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TABLE 1. RELEASE OF HISTAMINE FROM RAT PERITONEAL MAST CELLS BY RESERPINE

Expt.	Histamine release (%)*	Mast cells (× 1000)†	Histamine release (%)*	Mast cells (× 1000)†
	Mast cells 4·5%		Mast cells 30%	
	36	72	50	74
1	9	190	36	195
	0	575	9	570
	Mast cells 6·5%		Mast cells 36%	
	55	48	42	47
2	28	130	45	148
	0	350	14	425

* Cells were incubated in 1-ml volumes for 2 hr with 5×10^{-5} M reserpine phosphate. Release in control cells has been subtracted from all values. This release was 2, 5, 6 and 14 per cent for cell suspensions containing 4·5, 6·5, 30 and 36 per cent mast cells respectively.

† 100,000 Mast cells contained 2·2 and 2·8 μg histamine in experiments 1 and 2 respectively.

Under experimental conditions similar to ours, Moran and Westerholm[7] observed no release of histamine by reserpine. Assuming similar concentrations of histamine per mast cell in both studies, the high control levels of histamine they report indicate that they were using cell concentrations greater than those which, in the present studies, released no histamine upon exposure to similar concentrations of reserpine. Furthermore, there is the possibility of sex and strain differences; they used male Wistar rats and we, female Sprague–Dawley rats. Sprague–Dawley rats are said to be more sensitive than are Wistar rats to depletion of adrenal catecholamines by reserpine.[12]

The dependency of histamine release on mast cell concentration noted in the present work suggests that reserpine might be taken up or bound by the mast cells. The possibility of reserpine binding also has been suggested by others, e.g. Carlini *et al.*,[13] with respect to serotonin release from neoplastic mast cells.

The present studies add histamine in mast cells to the list of amines that are released by reserpine. Since peritoneal mast cells are easily obtained and manipulated, they might prove to be a convenient tissue for future studies on the mechanism of action of reserpine.

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