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Serotonin and Histamine: Effects of a Single Injection on the Mouse Testis and Prostate Gland.* (28306)

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Serotonin and histamine occur together in high concentrations in localized regions, such as in mast cells(1,2) and in the hypothalamus (3). These 2 compounds, classified as hormones, frequently have similar pharmacological effects as evidenced by studies on vascular permeability(4,5) and vasoconstriction (6,7). It has been suggested that serotonin may act as a synergist with histamine or that part of its action may result from a release of endogenous histamine(8). Recently, while examining organ systems in mice that had been given an injection of histamine and serotonin combined in a single dose, gross changes in the size and color of the prostate glands were observed. Following these preliminary observations, additional mice were treated identically to augment the data for this presentation.

Methods. Male Swiss Yale albino mice, 6 to 8 weeks of age at the beginning of the experiment, were injected subcutaneously with serotonin (*5HT*) and/or histamine (*H*) in 0.25 ml physiological saline. Dosages were fractions of the established effective dosage of serotonin creatinine sulfate and histamine diphosphate (CalBiochem.)(9,10). Group I, killed 7 days after a single injection, consisted of: (a) saline controls, (18 mice); (b) *5HT*, 6.25 mg/kg (8 mice); (c) *H*, 200 mg/kg (8 mice); (d) *5HT*, 3.13 mg/kg, plus *H*, 100 mg/kg (10 mice). Group II, killed 30 days after a single injection, consisted of: (a) saline controls (10 mice); (b) *5HT*, 3.13 mg/kg plus *H*, 100 mg/kg (10 mice); (c) *5HT*, 6.25 mg/kg, plus *H*, 200 mg/kg (10 mice).

At autopsy the testes and ventral prostate of each mouse were weighed and then fixed in Bouin's fluid. Sections were stained with hematoxylin and eosin, Mallory's trichrome stain, or PAS (periodic acid-Schiff). Organ weights were expressed in milligrams per 100 g of body weight (Table I).

Results. Prostate glands from mice that received either histamine or serotonin, or a combination of the 2 drugs, had mean weights which significantly exceeded those of the saline controls; the greatest increase was displayed following injection of the 2 drugs in combination (Table I). At 30 days mean weights of experimental prostate glands still were greater than controls; however, the only statistically significant change was in the group of 9 mice injected with 6.25 mg serotonin and 200 mg histamine per kg body weight. The most frequent histological changes in the prostates were an increased

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TABLE I. Weights of Prostate Glands and Testes of Mice Killed 7 Days and 30 Days After a Single Injection of Serotonin (5HT) and/or Histamine (H). Weights of glands are expressed as mg ± standard error per 100 g body weight. Dosages are in mg of the drug per kg body weight.

Dosage (mg/kg) 5HT:H	Prostate				Testis			
	7 day		30 day		7 day		30 day	
	No.	Wt	No.	Wt	No.	Wt	No.	Wt
0:0	18	33.41 ± 2.66	10	32.83 ± 2.40	18	560.97 ± 31.47	10	492.00 ± 26.95
6.25:0	8	41.38 ± 2.15*			8	495.38 ± 30.24		
0:200	8	42.83 ± 2.44†			8	522.75 ± 35.52		
3.13:100	17	68.27 ± 8.01‡	10	35.60 ± 3.06	17	543.18 ± 10.87	10	587.20 ± 57.86
6.25:200			9	45.64 ± 2.46§			9	465.56 ± 31.55

* $P < .05$ † $P = .02$ ‡ $P < .001$ § $P < .01$

size of intertubular spaces and enlarged tubules filled with dense PAS positive secretion. Venules of these organs were engorged with erythrocytes, and this might account for their deeper pink color which was observed prior to fixation. Glandular epithelium was absent from portions of the wall of some prostatic tubules (3 to 10 per section), while in others the lumen contained many spherical, acidophilic cells of undetermined origin. There was an exfoliation of cells from inner surfaces of prostatic ducts in treated mice. Comparison of prostatic secretion in fixed sections indicated that it was more often homogeneous in treated mice and not as granular in appearance as in the control group.

Testes from mice of every experimental group at 7 and 30 days displayed distinct and pronounced histological changes. Seminiferous tubules showed degrees of degeneration ranging from a rather minor change, such as pyknosis of spermatogonial cells in an otherwise normal tubule, to a striking absence of all cells excepting a single outer layer. Intermediate stages of tubular degeneration were characterized by exfoliation of cellular debris into the lumina of tubules or by the presence of scattered, larger vacuolar spaces, which possibly were the result of death and resorption of one or more spermatogenic cells. The lumen of a degenerating tubule frequently contained one to 3 large multinucleate cells. An extensive range in testicular weights existed among the individual mice of each category, and, although the means indicated alterations (Table I), weights of the treated testes were not statistically different from the control organs.

Discussion. There appear to be several possible sites for drug action in these animals, for example, direct effects on local vascular structures(4,5), on endocrine glands affecting the prostate glands and gonads, or on hypothalamic centers mediating the pituitary gland(11). The enlarged intertubular spaces, indicative of edema, and the engorged venules in the prostate glands of treated mice are characteristic of vascular leakage. The apparent return to normal weight of the prostates by 30 days may be due to recovery of normal structure and function in affected venules and to a reduction of edema. The most significant weight change in these organs, which occurred after injection of a combination of the 2 drugs, is additional evidence that the action of serotonin in mice may be the result of release of endogenous histamine(8) or may be synergistic to that of histamine.

The pathological changes in the treated testes are morphologically identical to those described following the occlusion of the testicular artery in rats(12). Boccabella *et al.*(13) reported decreased weight of the testes and prostate glands and similar histological changes in the testes after repeated injections of greater dosages of serotonin than used here; they concluded that their changes were due to vascular effects of the drug, and also that a single injection did not produce vasoconstriction of significant magnitude and duration in rats to cause pathological changes. The demonstration of continued testicular degeneration in treated mice at 30 days raises questions relating to duration and extent of

vascular damage from a single injection of these drugs, which are normally rapidly metabolized and excreted(14,15), and to the possibility of a localized neurotoxicity of the drugs which indirectly affect the endocrine system(16,17).

Summary. Two amines, serotonin and histamine, when administered either separately or in combination, caused weight increase in prostate glands and histological changes indicative of edema or vascular defect. Pathological changes in testes of treated mice included degenerating seminiferous tubules that contained spermatogonial cells with pyknotic nuclei and giant multinucleate cells. Absence of all spermatogenic cells from tubules was characteristic of advanced degeneration. It is suggested that histological changes in the testes were not necessarily the result of pharmacologic effects of the two amines on vascular structures.

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Metabolism of 9-Ethyl-6-MP-S^{35} and 9-Butyl-6-MP-S^{35} in Humans.* (28307)

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9-ethyl-6-mercaptopurine (9-E-6-MP) and 9-butyl-6-MP (9-B-6-MP) inhibit growth of the 6-mercaptopurine (6-MP) sensitive rodent tumor, adenocarcinoma-755(1). A 6-MP resistant strain of this tumor is cross-resistant to the 9-alkylated derivatives. 9-E-6-MP has also been demonstrated to produce remission when administered orally to chronic granulocytic leukemia patients(2). The 9-alkylated derivatives of 6-MP have been demonstrated to inhibit reproduction of cells in tissue culture(3), but cross-resistance between 6-MP and 9-alkylated derivatives was not observed when the number of carbons in the alkyl group was less than 5.

Evidence has been presented that 6-MP is converted to 6-MP-ribotide by cells sensitive to this drug(4,5,6). The ribotide of 6-MP has been postulated to be the "lethal" inhibitor of 6-MP sensitive neoplastic cells. We have found that the rat is not capable of dealkylating the 9-alkylated derivatives of 6-MP, a prerequisite step to formation of a ribotide from these compounds(7). It would, therefore, appear that free thiopurine might possess carcinostatic activity as has been suggested by Mandel(8). This report deals

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