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Received June 22, 1956. P.S.E.B.M., 1956, v93.

Some Observations on Nature of Refractoriness to Histamine Liberators.* (22653)

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The ability of a variety of agents including compound 48/80† to liberate histamine *in vivo* and *in vitro* has been well established. A phenomenon of acute tolerance development has been described for many of these agents and its mechanism is commonly ascribed to depletion of available body histamine stores by previous administration of the histamine liberator(1). The experiments here reported suggest the existence of an alternate mechanism of production of a refractory state to these histamine liberators.

Methods. Mongrel dogs were anesthetized with 33 mg/kg sodium pentobarbital. Arterial blood pressure was recorded from a common carotid artery and injections were made into a femoral vein. Serial blood samples were withdrawn in heparinized syringes from the other femoral vein in those experiments in which estimations of plasma histamine were made. Plasma histamine levels were determined by direct addition of the plasma to segments of guinea pig ileum suspended in atropinized Tyrode's solution, the contraction height being compared with those produced by known amounts of histamine. Pharmacologic identification of the stimulating agent as histamine was established by the inhibitory effect of Benadryl (final concentration $1:1x10^6$).

Two methods of administration of compound 48/80 were employed: (A) 200 μg/kg compound 48/80 was administered as a single rapid injection. (B) A series of graded doses beginning with 1.5 μg/kg and increasing to 200 μg/kg compound 48/80 was administered by slow injections spaced 10 to 15 minutes apart (cumulative dose of 596 μg/kg over a 3- to 5-hour period).

Dogs thus treated were challenged with 200 μg/kg compound 48/80 injected rapidly, and in several instances by 0.1 ml/kg of 10% Tween 20.

Results. The precipitous and prolonged hypotensive effect of a rapid injection of 200 μg kg compound 48/80 in the anesthetized dog (Fig. 1) qualitatively agrees with that

TABLE I. Plasma Histamine Concentrations before and after Administration of Compound 48/80.

Pre-treatment	No. of dogs	Plasma histamine conc. (μg/ml) Control	Min. after 200 μg per kg compound 48/80: 5	15-30
None	3	.13 ± .04*	.57 ± .10	.40 ± .05
596 g/kg, compound 48/80 †‡	4	Not detectable§	Not detectable	—

* Mean ± stand. dev.
† This amount of compound 48/80 was administered over a period of 3-5 hr in a series of graded doses ranging from 1.5-200 μg/kg.
‡ No histamine was detectable in plasma samples obtained every 30 min. during this period.
§ Sensitivity of the histamine assay method is estimated to be .05 μg histamine.

* This investigation was supported by research grant G-4077 (C) from the National Institutes of Health, Public Health Service.

† Compound 48/80 is a mixture of the dimers, trimers, and tetramers resulting from the condensation of N-methylhomoanisylamine and formaldehyde. Compound 48/80 was kindly made available to us by Dr. E. J. de Beer, The Wellcome Research Laboratories, Tuckahoe, N. Y.

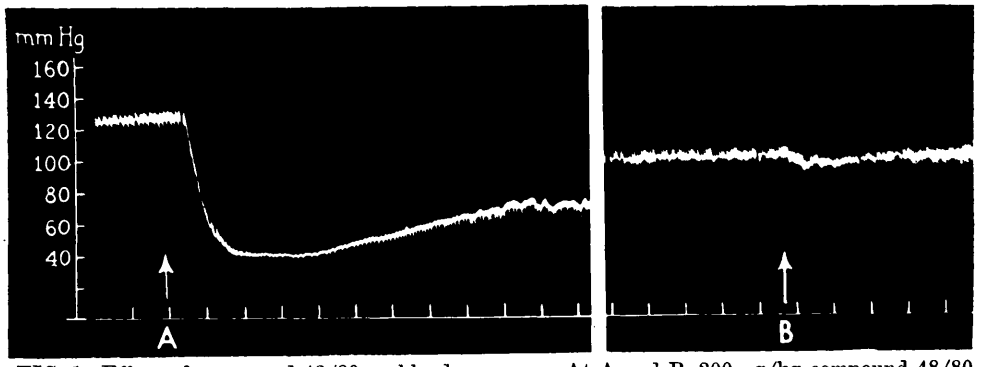

FIG. 1. Effect of compound 48/80 on blood pressure: At A and B, 200 μg/kg compound 48/80 was inj. rapidly. Base line signal interval, 1 min.; interval between sections, 69 min.

reported by others(2). An increase in plasma histamine concentration was found to occur in the early period of hypotension (Table I).

When the administration of compound 48/80 was modified to a series of graded doses ranging from 1.5 to 200 μg/kg, it was possible to inject a total of 596 μg/kg over 3 to 5 hours without producing changes in the blood pressure; these animals were subsequently refractory to the rapid injection of 200 μg/kg (Fig. 2). No changes in plasma histamine concentration were detectable during the period of administration of the graded doses or following the rapid injection of 200 μg/kg compound 48/80 in these animals (Table I).

Tween 20 produced a marked hypotensive effect in both groups of 48/80 refractory animals.

Discussion. The development of tolerance to the action of histamine liberators has been described in a variety of species. Feldberg and Paton(3) demonstrated the ability of various histamine liberators to deplete tissues of virtually all of their available histamine.

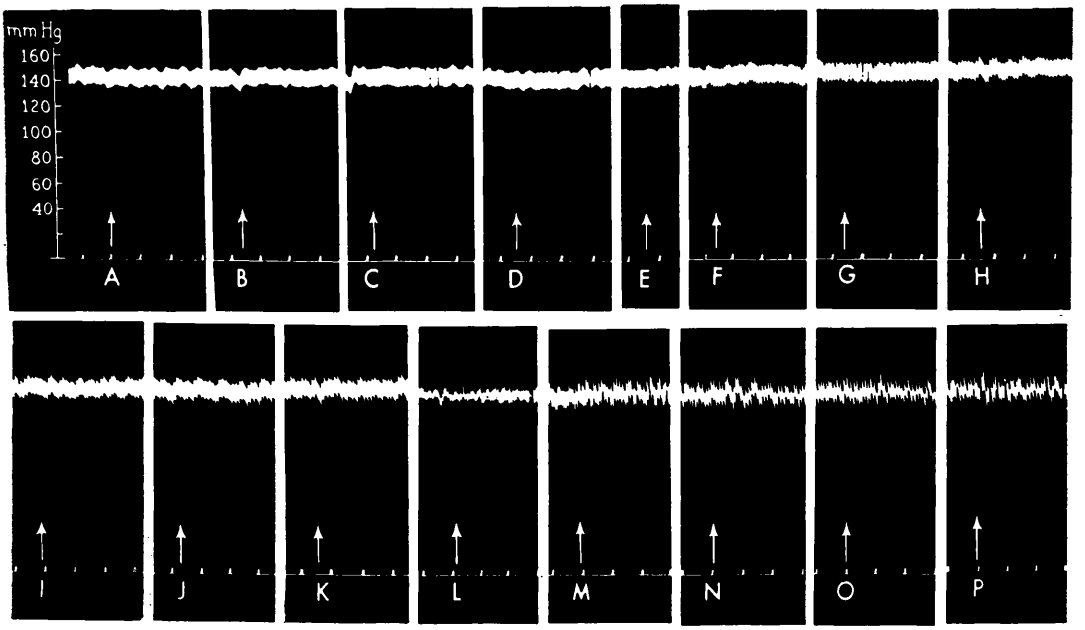

FIG. 2. Effect of compound 48/80 on blood pressure: Compound 48/80 was administered in a graded dosage schedule by slow injections as follows: At A and B, 1.5; C and D, 3.1; E and F, 6.3; G and H, 12.5; I and J, 25; K and L, 50; M and N, 100; O, 200 μg/kg. At P, 200 μg/kg was inj. rapidly. Base line signal interval, 1 min.; interval between successive injections, 5-10 min.

Sufficient histamine is released by compound 48/80 in the cat and dog to account for its hypotensive action, and refractoriness to this compound is associated with its inability to liberate more histamine(4). The experiments of Feldberg and Talesnik(1) indicate that refractoriness to compound 48/80 is associated with the depletion of available histamine body stores by prior administration of the histamine liberator. Mayeda(5) has demonstrated cross-tolerance to the histamine liberating action of peptone and sinomeminine, and Slomka and Goth(6) have demonstrated a similar cross-tolerance between compound 48/80 and several morphinan compounds. This cross-tolerance may also be explained on the basis of depletion of available histamine body stores. Indeed, the marked reduction in the edema produced by light in porphyrin-treated rats whose skin histamine had been markedly depleted by prior administration of compound 48/80(1) lends support to this hypothesis. Although depletion of available histamine stores provides an obvious mechanism for the development of tolerance to histamine liberators, the results of our experiments suggest that an alternate mechanism of inducing a refractory state exists. Since no increase in circulating histamine concentrations was detectable during the development of the refractory state to compound 48 80 with a graded dosage schedule, it appears unlikely that a significant amount of histamine was liberated, *i.e.* enough to explain the refractory state by depletion of the available histamine body stores. The responsiveness of the cardiovascular systems of such 48/80 refractory dogs to exogenous histamine is unaltered. The ability of the histamine liberator Tween 20 to produce its characteristic hypotensive effects in 48/80 refractory animals further suggests that the depletion of available histamine body stores has not been accomplished. The ability of compound 48/80(7) and Tween 20(8) to liberate histamine from liver mitochondria has been demonstrated.

Although its mechanism has not been elucidated, an interpretation of our results suggest that acute refractoriness to the histamine liberator compound 48 80 has been developed in dogs by means other than that of depletion of available body stores of histamine.

Summary. The administration of a series of graded doses of compound 48/80 produces a refractory state to the histamine liberating action of this compound. This refractory state appears to be effected by a means other than that of depletion of available histamine body stores.

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Received June 27, 1956. P.S.E.B.M., 1956, v93.