Properties of Seizures Induced by Histamine.* (24440)

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Presence of histamine in the central nervous system has been demonstrated(1) but its functional significance is not understood. In previous studies, effects of histamine on the brain have been examined in laboratory animals and responses have varied with method and site of injection. When the drug was administered through a cannula implanted in the lateral ventricles, Feldberg and Sherwood (2) observed profound muscular weakness and signs of autonomic disturbance in the unanesthetized cat. By direct intracerebral injection of histamine in the mouse, however, Haley(3) has referred to the occurrence of clonic convulsions in addition to other toxic manifestations. That histamine may be involved in the seizure mechanism is suggested by some central actions of antihistaminics and the efficacy of these agents in the control of certain seizures. Diphenhydramine is effective in treatment of petit mal epilepsy; it will eliminate from the electrocorticogram seizure discharges experimentally induced in animals (4). Central sympathetic stimulation and a pressor response obtained by injection of histamine into the lateral ventricle of the cat were abolished by previous injection of an antihistaminic in this site(5). Further, a remarkably high incidence of allergy has been observed in children with autonomic(6) and febrile seizures(7) and attention has been drawn to a possible relation between allergy and occurrence of abnormal electroencephalographic patterns(8). The present laboratory investigation was designed to elucidate further the relation of histamine to seizures. Properties of histamine-induced seizures and their modification by various classes of drugs have been studied in both mice and guinea pigs, since these animal species differ markedly in their sensitivity(9,10).

Methods. Swiss albino mice weighing 18-22 g, and young guinea pigs, 100-200 g, were used as experimental animals. The intracerebral dose of histamine acid phosphate required to produce convulsions in 97% of animals was established (CD_{97}) and the pattern and duration of the seizure examined. Histamine, administered by the method of Haley and McCormick(11), was injected to a depth of 2-3 mm at a point 2 mm from the midline of the skull at the level of the anterior base of the ears. All doses are stated in terms of the salt. A syringe graduated to 0.001 ml and 3/8", 30 gauge needle facilitated accurate measurement and administration of small quantities of drug; occurrence of leakage along the needle track, as pointed out by Bedford(12), was reduced to a minimum. Possible reactions due to penetration and trauma of the brain by the hypodermic needle or volume of fluid *per se* were investigated. Effects of distilled water and isotonic saline were compared with those of phosphate buffer solutions of various pH values. Since histamine acid phosphate in isotonic saline was markedly acidic, the drug was administered in phosphate buffer solution at pH 5.25. Anticonvulsants, tranquilizing agents, antihistaminics and adrenocortical steroids were tested for ability to abolish or modify the pattern of seizures induced by the CD_{97} of histamine. In mice, except for antihistaminics administered by the intraperitoneal route in one experiment and adrenocortical steroids which were injected subcutaneously, all drugs were given orally and dissolved or suspended in 10% acacia solution, of approximate volume 0.4 ml per 20 g body weight. In guinea pigs, drugs were administered by the intraperitoneal route, with the exception of serpasil which was given orally; the fluid volume injected was approximately 0.5 ml per 100 g body weight. Five animals were tested at

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each dose level and responses compared with a control group given histamine alone. Apart from adrenocortical steroids, drugs were examined for time of peak activity. For determination of anticonvulsant potency, groups of 5 mice given graded amounts of drug were challenged with the CD_{97} of histamine at the time of peak effect, until points were established between complete and no protection. Results were plotted on logarithmic probability paper and a regression line drawn. From these data, the ED_{50} was determined and 95% confidence limits calculated. The anticonvulsant ED_{97} in mice was used for tests of anticonvulsant drug action in guinea pigs. Anticonvulsant and antilethal effects of tripelennamine, diphenhydramine and chlorpromazine were compared in guinea pigs and mice; 5 animals were challenged with an intracerebral CD_{97} of histamine at one half hour after intraperitoneal administration of a 25 mg/kg dose of each antihistaminic. In addition to observations of the convulsant action of histamine when injected intracerebrally, effects of intraperitoneal injection were investigated in mice subjected to electroshock and pentylenetetrazol (metrazol) seizures. The threshold to maximal electroshock seizures in m.amps. was determined 45 min. before and 5 min., 1 and 2 hr subsequent to injection of histamine in doses of 5-2000 mg kg. A group of control mice was tested at the same time intervals. Similar doses of histamine were administered to mice given a CD_{97} of metrazol (85 mg/kg) by subcutaneous injection after an interval of 5 min. Time of onset and severity of the seizure were compared in control animals not treated with histamine.

Results. Induction of seizures by intracerebral histamine. A momentary decrease in motor activity followed penetration of the brain with the needle and injection of a volume of distilled water or isotonic saline, 0.01 ml in the mouse and 0.02 ml in the guinea pig; convulsions did not result. No untoward reactions occurred in animals injected intracerebrally with solutions of M/15 KH_2Po_4 and Na_2HPo_4 at pH values from 4.52 to 8.83. Histamine acid phosphate, 200-500 γ in 0.01 ml of isotonic saline, had a pH value of 3.9. When dissolved in a phosphate buffer of pH 8.2, the pH of histamine solution was 5.25. Histamine, 200-500 γ (10-25 mg/kg) by intracerebral injection in the mouse, produced the following seizure pattern: onset within 3-5 min. with circling and rolling toward the side of injection; hyperkinetic behavior and tremor were followed by intermittent clonic jerks and flexor and extensor spasms of fore- and hind-limbs; severe generalized clonus, the principal component of the seizure, was repeated at irregular and short intervals and was succeeded by tonic extension of all limbs and death of the animal within 5-30 min. Occasionally, tonic extension of hind-limbs occurred at the onset of the seizure. The CD_{97} of intracerebral histamine in the mouse was 25 mg/kg; at doses below 25 mg/kg but greater than 10 mg/kg, occurrence of convulsions was less constant. Injection of 0.25-10 mg/kg of histamine caused tachypnea, defecation, urination, vasodilation and irritation of the ears and occasionally, a Straube tail phenomenon; convulsions did not occur. In the guinea pig, a clonic seizure pattern similar to that in the mouse was observed following intracerebral injection of histamine in doses of 5-10 mg/kg. A dose of 10 mg/kg produced seizures consistently and was used in subsequent tests of anticonvulsant activity of drugs. Compared to the mouse, occurrence of convulsions and death was more rapid; generalized clonus began within 10 to 60 sec. and the animals died in 1 to 4 min. A typical seizure in the guinea pig consisted of cycling movements of fore-limbs followed by generalized clonus; tonic extension of hind-limbs was noted rarely. While the convulsive and lethal intracerebral dose of histamine in the guinea pig was smaller than in the mouse, the difference in sensitivity was considerably greater when histamine was injected intraperitoneally. By this route of administration, the LD_{97} in the guinea pig and mouse was 10 and 2500 mg/kg, respectively.

Actions of drugs on histamine-induced seizures. Anticonvulsants. (Table I). Trimethadione and phenobarbital abolished seizures completely and the animals survived; protective or therapeutic indices in the mouse compared favorably with those reported in

TABLE I. Effect of Anticonvulsants against Seizures Induced by Intracerebral Histamine.

Drug	Time of maximal effect, hr	Effective $dose_{50}$, mg/kg	Protective index*
Trimethadione	1½	295 (204–428)	2.3
Phenobarbital	3	30 (20– 44)	1.9
Acetazolamide	3	630† (460–860)	3.2
Diphenylhydantoin	3	—‡	0

* Ratio TD_{50}/ED_{50}. † Incomplete control.
‡ Seizures exacerbated.

tests of anti-metrazol activity(13). In the sedative doses employed, both trimethadione and phenobarbital were effective also against histamine seizures in the guinea pig. Respective values for the ED_{50} were not determined in this species. The depressant effect of trimethadione was remarkable in the guinea pig and much greater than in the mouse. In mice, acetazolamide in non-toxic doses prevented severe clonic seizures and death in tonus; hyperkinesia, circling movements, and mild clonic jerks were not abolished, even with doses of 1800 mg/kg. In guinea pigs, the partial anticonvulsant action of acetazolamide observed in mice was not demonstrated at a dose level of 1800 mg/kg. Diphenylhydantoin, 25-300 mg/kg in mice and 50 mg/kg in guinea pigs, failed to prevent and indeed, exacerbated histamine-induced clonic seizures; the small tonic component of the seizure was abolished but death ensued.

Tranquilizers. Chlorpromazine, in oral doses of 5 and 50 mg/kg, had no anticonvulsant or antilethal action in mice tested with histamine intracerebrally after an interval of 1 hr. In guinea pigs given chlorpromazine intraperitoneally and tested at ½ hr, death from intracerebral histamine was prevented by a dose of 5 mg/kg and the seizure was obtunded in addition in animals treated with a dose of 25 mg/kg. Injection of histamine was repeated after 2 hours in 5 animals given the larger dose of chlorpromazine; 2 animals exhibited generalized clonic seizures of delayed onset but all survived. Both mice and guinea pigs were sedated by the larger doses of chlorpromazine employed. In addition to a protective effect against histamine administered intracerebrally, chlorpromazine, in a dose of 25 mg/kg, prevented convulsions and death in guinea pigs given 3 lethal doses of histamine (30 mg/kg) by the intraperitoneal route. Mice treated with the same dose of chlorpromazine were not protected from 2 lethal doses of histamine (6000 mg/kg intraperitoneally) and, compared with controls, the time of death was accelerated. Serpasil, in sedative doses of 20 and 40 mg/kg, caused exacerbation of seizures in both mice and guinea pigs; animals were challenged with histamine at 1½ hr after administration of drug.

Antihistaminics. In mice tested at 1, 2 and 3 hr after oral administration of drug, tripelennamine 50-150 mg/kg, diphenhydramine 25-50 mg/kg, and thephorin 100-200 mg/kg failed to protect against histamine-induced seizures and death. Tripelennamine, in toxic doses of 200 and 250 mg/kg, prevented convulsions and death in 2 of 4 mice tested with intracerebral histamine; larger doses of 400-500 mg/kg had convulsant and lethal effects. Diphenhydramine, in 100, 200 and 300 mg/kg doses and thephorin, 400 and 500 mg/kg, caused hyperkinesia, convulsions and death in tonus. In guinea pigs tested at 10-15 min. after intraperitoneal administration of drug, antihistaminics showed protective effects. Tripelennamine, in a non-toxic dose of 5 mg/kg, prevented death and reduced severity of histamine-induced seizures. At a dose of 10 mg/kg, both tripelennamine and diphenhydramine abolished the generalized clonic component of the seizure in 4 of 5 animals tested. Of the 5 guinea pigs treated with diphenhydramine and given a second injection of histamine after an interval of 1 hr, generalized clonic convulsions occurred in 4 and tonic extensor spasms of both fore- and hind-limbs were associated in 2. In the experiment designed to compare the relative potency of effect of antihistaminics in guinea pigs and mice, equal and non-excitant doses of tripelennamine, diphenhydramine and the weak antihistaminic, chlorpromazine showed greater and and more specific anticonvulsant and anti-lethal activity in the guinea pig (Table II). Apart from tripelennamine, which pro-

TABLE II. Comparison of Effects of Antihistaminics in Guinea Pigs and Mice Treated with an Intracerebral CD_{97} of histamine.

Antihistaminic (25 mg/kg)	Anticonvulsant potency*		Anti-lethal potency*	
	Guinea pig	Mouse	Guinea pig	Mouse
Tripelennamine	2	1	4	1
Diphenhydramine	3	0	5	2
Chlorpromazine	3	0	3	2
Total	8/15 (53%)	1/15 (7%)	12/15 (80%)	5/15 (33%)

* Expressed as No. of animals out of 5 protected.

tected only 1 of 5 mice from histamine-induced seizures, antihistaminics were ineffective as anticonvulsants in this animal species. Indeed, in mice treated with diphenhydramine, the onset of convulsions was accelerated and clonus was exacerbated.

Adrenocortical steroids. Cortisone acetate, in a dose of 140 mg/kg daily, failed to prevent and accelerated convulsions and death in 10 mice tested after treatment for 4 consecutive days. Desoxycorticosterone acetate, 27 mg/kg administered daily for 4 days, also failed to prevent, but retarded the onset of seizures and death. The threshold to minimal electroshock seizures is affected similarly by adrenocortical steroids(14) and, like histamine-induced seizures, the minimal electroshock seizure though less severe is principally clonic in type. Histamine given intraperitoneally in doses of 5, 50, 500 and 2000 mg/kg delayed the onset of metrazol-induced seizures by 3.6, 2.2, 6.1 and 24 min., respectively. In 10 control mice, mean time of onset of clonus was 2.9 min. (with a range of 1.5-4.5); in 4 groups of 5 histamine-treated mice, clonus occurred at 6.5, 5.1, 9 and 27 min., respectively. Generalized clonic seizures were prevented in 1 of 5 animals given histamine in a dose of 500 mg/kg and in a similar number treated with a toxic dose of 2000 mg/kg. Histamine, by intraperitoneal injection in the same dose range, had no significant effect on the maximal electroshock seizure threshold in mice.

Discussion. When administered intraperitoneally, histamine will induce seizures fairly consistently in the guinea pig but less commonly in the mouse. In response to intracerebral histamine, however, seizures are the most remarkable manifestation of toxicity and their properties may be compared in both animal species. Histamine-induced seizures are principally clonic in type and the pattern resembles that produced by metrazol and by fever(7). They are prevented by trimethadione, an antiepileptic drug which controls experimental metrazol and febrile seizures, and are exacerbated by diphenylhydantoin, which is ineffective against metrazol and fever-induced seizures(7). Whereas trimethadione is of value clinically in treatment of petit mal and minor clonic seizures, the incidence of such seizures may be increased by diphenylhydantoin(4). Evidence of a diencephalic origin for petit mal(15) and possibly for febrile seizures(16) has been reported. Further, it is of interest that relatively high concentrations of histamine have been found in the region of the hypothalamus(1). These observations, together with the known efficacy of antihistaminics in treatment of petit mal, suggest that paroxysmal and excessive release of histamine in the diencephalon may possibly be involved in the origin of certain seizure discharges.

That histamine may be involved in the seizure mechanism is supported further by the anticonvulsant action of antihistaminics observed in laboratory animals. In the guinea pig, an animal highly sensitive to histamine, tripelennamine and diphenhydramine are antagonistic to histamine-induced seizures whereas in the mouse, a histamine-resistant species, antihistaminics have little or no protective effect. The high natural resistance observed when histamine is administered parenterally in the mouse is not understood; it may be explained by a relatively rapid rate of inactivation by acetylation, cellular binding and the enzyme histaminase. The minimal signs of toxicity to the central nervous system may be due to low concentrations of drug in the brain. The difference in sensitivity between mouse and guinea pig is much less remarkable when histamine is given by direct intracerebral injection. While pertussis vaccine is known to enhance sensitivity of the mouse to histamine given intraperitoneally (17,18) an exacerbation of convulsions in-

duced by histamine given intracerebrally could not be demonstrated.§ However, despite the greater uniformity in sensitivity to intracerebral histamine, the guinea pig and mouse differ markedly in response to antihistaminics. In addition to their ineffectiveness against histamine-induced seizures in the mouse, antihistaminics fail to protect this species from a lethal parenteral dose of histamine. In the mouse, toxic effects of antihistaminics are added to those of histamine (19) whereas in the guinea pig, protective effects are evident. The ability of chlorpromazine to obtund histamine seizures in the guinea pig but not in the mouse may be related to its weak antihistaminic activity. Serpasil, a tranquilizing agent without antihistaminic properties, exacerbated histamine seizures in both mice and guinea pigs.

The guinea pig differs from the mouse and rat, not only in its sensitivity to histamine but in the seizure response to electroshock stimuli. A maximal seizure with tonic extension of hind-limbs is observed rarely and generalized clonus is the principal component (20). Since histamine-induced seizures are mainly clonic in type and certain clinical convulsive disorders may possibly be related to abnormal histamine release, the guinea pig should prove of value in laboratory evaluation of potential anticonvulsant compounds.

Libet *et al.*(21) have investigated a possible *anticonvulsant* action of histamine given parenterally in dogs. Seizure discharges induced by leptazol were prevented or their onset was delayed. The apparent anticonvulsant effect was explained by histamine-induced vasodilation and consequent dilution of leptazol. The present experiments in mice confirm these observations. While metrazol-induced seizures are retarded or abolished by histamine injected intraperitoneally, the threshold to electroshock seizures is not modified significantly. Thus, a non-specific antagonism to metrazol and seizures is suggested. Furthermore, in clinical studies, histamine failed to control petit mal seizures (22) and had no demonstrable effect on the electroencephalogram(23).

§ Personal observation (R.K.).

Summary. Clonic seizures were induced in the mouse and guinea pig by intracerebral injection of histamine. They were prevented by trimethadione, exacerbated by diphenylhydantoin, and incompletely controlled by acetazolamide. Apart from chlorpromazine, tranquilizing agents were ineffective. Cortisone and desoxycorticosterone caused mild exacerbation and some reduction in severity of seizures, respectively. Antihistaminics prevented histamine seizures in the guinea pig but not in the mouse. The difference in sensitivity of these species to histamine by intraperitoneal injection was less evident by the intracerebral route. Control of metrazol-induced seizures by histamine was a non-specific antagonism. The possible relation of histamine to seizure mechanisms is discussed.

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Comparative *in vitro* Corticoidogenic Effects of Rat Adenohypophysial Extract and U.S.P. Reference Standard Corticotropin.* (24441)

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Consistently higher values for pituitary ACTH concentration, in the intact rat, have been obtained with Saffran and Schally's *in vitro* corticoidogenic assay(1) than with Sayers' *in vivo* adrenal ascorbic acid depletion method(2). Due allowances being made for the different strains of rats and extraction procedures utilized by various groups, the values recorded, in terms of milliunits (mu) of ACTH/mg of fresh pituitary tissue, range from 10 to 18(3-6) with Sayers', and from 52 to 241(7,8) with Saffran and Schally's assay. A mean concentration of 64.6 ± 1.8 mu/mg was recorded, in this laboratory, from 100 *in vitro* assays (average λ : 0.106 ± 0.004) of 0.1N HCl-extracted pituitaries from intact male Sprague-Dawley rats (Fortier, unpublished). To account for the discrepancy in results yielded by the 2 assaying procedures, the possibility was raised by Birmingham *et al.*(7) that *steroidogenic/ascorbic acid depleting* (S/AA) activity ratio might be higher in native (pituitary extract) than in more purified (Reference Standard) corticotropin. This view was not supported by results of concurrent 4-point-2-dose assays of the same pituitary extract against the same standard, respectively based on *in vitro* corticoidogenesis, and on adrenal ascorbic acid depletion and rise of adrenal and plasma corticosteroids in hypophysectomized rats (Guillemin, Fortier and Lipscomb, unpublished). Nearly identical values (20-24 mu/mg) were obtained on the basis of *in vivo* corticoidogenesis and ascorbic acid depletion, whereas a markedly higher concentration (62 mu/mg) was again recorded on the basis of *in vitro* corticoidogenesis. The discrepancy would thus appear to be related to differences in the nature (*in vivo* or *in vitro*) rather than in the end-points (adrenal ascorbic acid depletion or corticoidogenesis) of the assays. The following possibilities were accordingly considered in the present study: a) Conditions prevailing in the *in vitro* assay might favor activation of a structurally related, hypothetical precursor of ACTH (*protocorticotropin*), contained in rat pituitary extract; b) Differences in the fates of, or responses to, the presumably distinct molecules corresponding to rat and hog (U.S.P. Reference Standard) corticotropins might be selectively enhanced by conditions prevailing in one of the 2 assaying systems under consideration. These possibilities were assessed by comparing rat pituitary extract and U.S.P. Reference Standard Corticotropin, in Saffran and Schally's *in vitro* system, with respect to inactivation, corticoidogenic effects, and extent of corticotrophic activation conferred to the incubated glands, as a result of a limited period of contact.

Materials and methods. Pituitary extracts. Adenohypophyses from adult male Sprague-

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