Pharmacological Differentiation of Allergic and Classically Conditioned Asthma in the Guinea Pig

Abstract. *A whole-body plethysmographic technique was developed and then used to detect experimentally induced asthma in guinea pigs and to assess pharmacological treatments of allergic and classically conditioned attacks. Inhalation of a beta adrenergic compound (isoproterenol) controlled both forms of attack. Atropine and methscopolamine, parasympathetic blocking agents, prevented conditional but not allergic attacks; diphenhydramine, an antihistamine, prevented allergic attacks; and methysergide, which blocks serotonin (which is believed to trigger human asthma), prevented neither. The guinea pig's allergic reaction is probably the result of a bronchospasm induced by histamine released in tissue of the airway by a local combination of allergen and antibody. The conditional attack is believed to be a constriction of the airway mediated by parasympathetic fibers of central origin.*

Allergic bronchial asthma rarely develops spontaneously in infrahuman animals (*1*), but it is readily produced experimentally in the guinea pig (*2*). When sensitized by forced inhalation or systemic infusion of a foreign protein and later provocatively challenged by it, the animal develops a bronchoconstrictive reaction that resembles the clinical attack (*3*). Moreover, the reaction is amenable to classical conditioning (*4, 5*). We report here studies bearing on the neuropharmacology of allergic and classically conditioned attacks of asthma in *Cavia porcellus*.

Previous investigators of classically conditioned asthma in the guinea pig have relied upon gross visual inspection to index attacks (*5*). Because mere confinement of the animal in a small conditioning chamber evokes behaviors such as pilomotor activity, licking and chewing, and rapid, jerky movements of limbs and body which also accompany experimental asthma (*6*), we developed a measure of asthma based upon whole-body plethysmography, which is relatively insensitive to behavioral confounding. The labored breathing characteristic of an asthma attack is due to increased impedance of the airway arising from congestion or constriction. Increased impedance will augment the positive intrapulmonic pressure that normally occurs during expiration and likewise will augment the partial intrapulmonic vacuum that occurs during inspiration. The pressure differential between inspiratory peak and expiratory peak of each respiratory cycle is thus greater during an attack of asthma than when the airway is patent. In whole-body plethysmography an animal in an airtight chamber surrounded by a fixed volume of air will, in generating the intrapulmonic pressure differential, cause a simultaneous and inverse change of pressure in the closed air space of the chamber.

The plethysmographic apparatus consisted of a hollow steel probe of a Statham P23BB pressure transducer inserted through a rubber seal into the interior of a chamber fabricated from ¼-inch (about 6-mm) Plexiglas sheet (internal dimensions: 31 by 15 by 13 cm). The chamber's base and walls were formed into a single unit, a continuous recessed lip near the inside top providing a seat for a removable lid. When the lid was in place and weighted by a standard concrete building block, the chamber could be rendered airtight. Two rubber hoses made of standard surgical tubing were connected with the chamber near the base; one was coupled to a filtered vacuum line, the other to an air supply metered to provide a pressure of 0.4 to 0.6 atm. A third hose could alternately be coupled to the air supply and was fitted to an Adrenomist nebulizer by which solutions were introduced into the chamber as aerosols. During recording of plethysmograms all three hoses were closed with hemostats. The output of the transducer was coupled to a Grass 6A1-1 d-c bridge and amplifier unit, thence to a power amplifier and widetrack ink-writing pen of a Grass 6-8-2 polygraph. Amplifying circuitry was calibrated to produce a vertical deflection of 1 cm/mm-Hg.

To determine whether changes of plethysmographic amplitude accurately reflected changes of airway impedance, we treated five adult guinea pigs with drugs known to cause (urecholine chloride) or to counteract (isoproterenol) constriction of the airway (*7*). After recording a basal plethysmogram we injected each of the animals subcutaneously with 200 μg of urecholine in solution (Bethanol at 5 mg/ml); three- to fivefold increases over basal plethysmographic amplitudes were subsequently observed (*8*). Within 1 to 2 minutes of an observed increase, the air line to the nebulizer and the vacuum return were opened and isoproterenol (Isuprel, 1 : 100) was introduced into the chamber as an aerosol for 3 minutes at a rate approximating 0.5 ml/min. Another plethysmogram was then recorded for each of the individually tested animals; amplitudes of all five had returned to basal levels (Fig. 1a).

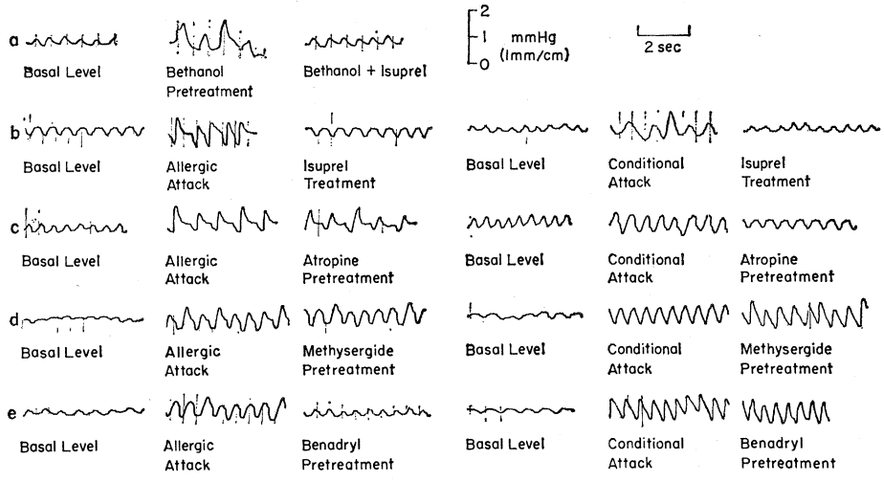

Fig. 1. Records typifying control (basal) and experimental plethysmograms of guinea pigs. (a) Three recordings generated by a normal animal. They reflect, from left to right: basal activity, response to an injection of Bethanol (urecholine), and response to Isuprel (isoproterenol) inhaled a few minutes later. The three records on the left in (b) through (e) are those taken during basal measurements, during allergic attacks, and after pharmacological treatment of the attacks, respectively. Each set of records is based upon one animal. The corresponding records on the right are from animals with classically conditioned attacks of asthma. Recordings from animals treated with methscopolamine closely resemble those of subjects treated with atropine.

Table 1. Summary of pharmacological treatments and their effects upon allergic and classically conditioned attacks of asthma. Each + sign represents an instance in which a drug controlled an attack; a — sign indicates absence of control; and a ? sign represents an instance of inconclusive control.

Drug	Dosage	Administration	Time before assessment (min)	Animals tested (No.)	Replications (No.)	Control exerted over	
						Allergic attack	Conditional attack
Isuprel	1 : 100	Inhalation	0	4	3	+++ +++ +++ +++	+++ +++ +++ +++
Atropine	5 mg/kg	Intraperitoneal	30	3	3	— — — — — —	+ + + + ? + + +
Methscopolamine	8 mg/kg	Intraperitoneal	30	3	3	— — — — — —	+ + + + + + + ?
Methysergide	10 mg/kg	Intraperitoneal	120	3	3	— — — — — ? —	— — — — — —
Benadryl	1 mg/kg	Intraperitoneal	30	10	2	++ ++ ++ ++ ++ ++ ++ ++ ++ ++	— — — — — — — — — — — — — — — — — — — —

Thirty-three experimentally naive guinea pigs (350 to 750 g) were then assigned to one of four treatment groups, one experimental and three control. Each of 23 experimental animals was injected intraperitoneally with 1 mg of crystalline egg albumin (purified five times and supplied by the Nutritional Biochemicals Corp.) dissolved in 1 ml of distilled water. Ten to 14 days later each animal was individually challenged for 3 minutes by an aerosol of the albumin in distilled water (800 mg/100 ml) delivered at a rate of about 0.5 ml/min. Three of the ten control animals were treated with distilled water only (that is, were injected with and then challenged by water); four were injected with water and then challenged by the protein; and the other three were injected with the protein and then challenged with water (sham challenge). None of the control animals generated plethysmographic changes to challenging treatments, whereas amplitudes increased at least threefold for 16 experimental animals; each of the remaining 7 convulsed and was dying, presumably from anaphylactic shock, before a plethysmogram could be recorded (*9*). Without being removed from the chamber, each surviving animal was exposed for 3 minutes to an aerosol of isoproterenol (1 : 100). Another plethysmogram was then recorded; without exception amplitudes had returned to basal levels.

Classical conditioning of allergic asthma was then attempted. Sixteen male guinea pigs (500 to 925 g), including eight used in the previous study, were selected for their reliability in responding with plethysmographically indexed attacks of asthma to three consecutive provocative challenges. Three experimentally naive animals were assigned to each of the three control conditions. All 25 animals were subjected daily to a series of six trials in the plethysmographic chamber. The first trial of each day consisted of a 3-minute challenge with water (sham challenge) that followed and was followed in turn by an 8- to 12-second plethysmographic measure (*10*). During each of the five ensuing trials a given animal was challenged by water or protein depending upon its control or experimental status. The aerosol of water or protein was released into the chamber for 3 minutes at 0.5 to 0.7 ml/min. The vacuum return as well as the positive-pressure line was operative throughout a challenging treatment; the nebulizer was then inactivated by diversion of the positive-pressure line to the chamber's air inlet. The resulting inrush of fresh air served to flush aerosol from the chamber. The animal was then removed and wiped with a clean, damp towel; the chamber was washed in distilled water, then in 95 percent ethanol, and again in distilled water. Forty-five to 60 minutes elapsed between trials of a daily series; during the intervals between trials the animal was housed in a carrying cage and removed from the conditioning area. Between daily series of trials animals were individually housed in their home cages.

In the procedure as described, an experimental animal (one sensitized to and then provocatively challenged by the protein) would generate an increase of plethysmographic amplitude and thus an unconditional asthmatic reaction (UR). Inhalation of the protein constituted presentation of the unconditional stimulus (US), and all other presentments of a challenging treatment (hissing sounds of the nebulizer, visual and tactile properties of the aerosol, and so forth) constituted the conditional stimulus (CS). The initial sham challenge of each daily series of challenging trials functioned as the test CS; a plethysmogram recorded before and immediately after the trial, respectively, yielded the base line and the measure of conditional responding (CR). Criterion for a CR was an increase in the average peak-to-peak amplitude of the plethysmogram equal to or greater than 2.5 times an animal's basal average (*11*).

Three experimental animals met criterion to the test CS on the 2nd day of conditioning; seven on the 3rd day; and the remaining six on the 4th day. None of the control animals generated noticeable increases across the 4-day span. When an experimental animal exhibited a CR it was given a series of sham challenges at 45- to 60-minute intervals until a plethysmogram, one being recorded after each challenge, registered an amplitude at the basal level. Resistance to extinction was moderate, CR's persisting from three to eight trials. Within 24 hours each experimental animal was provocatively challenged five times at 45-minute intervals and then presented a test CS; all exhibited recovery of the CR.

To gain information on nervous (*12*) and humoral pathways participating in allergic and conditional attacks, we treated ten of the experimental animals with several drugs (Fig. 1 and Table 1). At least three animals were treated with a given drug for an allergic attack, the same three animals also receiving the same treatment on another occasion for a conditional attack; in addition, each treatment of a given animal was repeated at least once and usually twice. The drug treatments were ordered across time so that the sequence of testing allergic and conditional reactions was random and drug effects could dissipate before retesting. In each test of a drug a basal plethysmogram was first recorded; next, control challenges were presented (sham or provocative or a sequence involving both) to confirm through generation of a plethysmogram of higher amplitude that an allergic or conditional reaction was intact (*13*); then the animal was subjected to a sham or provocative challenge after being treated with the drug. If the amplitude of a plethysmogram recorded after the challenge fell to the basal level, an instance of positive control was re-

corded; if an intermediate fall occurred, an instance of inconclusive control; if amplitude remained high, no control.

The rationale for selection of drugs was primarily based upon reference materials in Goodman and Gilman (*14*). We anticipated (i) that inhaled isoproterenol would control both allergic and conditional attacks; (ii) that a conditional attack, presumably parasympathetically mediated, would respond to cholinergic blocking agents possessing both central (atropine as a sulfate) and peripherally restricted (methscopolamine as a bromide) sites of action; (iii) that methysergide, a peripherally acting congener of LSD and a potent blocker of serotonin, would prevent allergic attacks; and (iv) that an antihistamine (Benadryl, diphenhydramine hydrochloride) would prevent neither form of attack. Although the first two expectations were confirmed, the last two were not.

Immunologists generally agree that allergic asthma occurs when an allergen and a specific antibody engendered by it combine in some fixed tissue of the airway. The combination releases a chemical irritant that constricts smooth muscle of the airway. Although histamine is implicated as an irritant of tissues in other hypersensitivities such as urticaria and angioedema, its importance as a bronchoconstrictive trigger in human asthma has been minimized; the role of trigger has been ascribed to other agents such as serotonin (*15*). The failure of methysergide to prevent allergic attacks and the implication that histamine and not serotonin is the guinea pig's bronchoconstrictive trigger suggest that asthmas in man and guinea pig are precipitated by a different chemical irritant. The data are open, however, to another interpretation. Asthma in the human being, at least when failing to respond to antihistaminic premedication, may be the classically conditioned aftermath of earlier provocations by an allergen. Perhaps histamine is a bronchoconstrictive trigger in early stages of human asthma; perhaps, too, the strong emotional states that usually accompany an attack of asthma are subject to classical conditioning. If so, their subsequent evocation in the absence of allergenic stimulation may be paralleled by activation of vagal or other parasympathetic pathways investing smooth muscle of the airway. This alternate interpretation is fully consonant with recently reported findings based upon human beings (*16*). Increased impedance of the airway as measured by whole-body plethysmography occurred in 13 of 29 chronically asthmatic patients when challenged by physiological saline. This psychogenic constriction of the airway, which was repeatedly but selectively occasioned when the patient believed he was being challenged by his allergen, was selectively abolished by controlled injections of atropine.

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8. The amplitude of basal plethysmograms was highly consistent for a given animal and usually was higher in the case of heavier animals. Because of this individual variation, absolute pressures were not used as criteria of changed airway impedance. Instead, the basal amplitude for each animal was used as the control reference for a plethysmogram generated during an experimental treatment.
9. Although all sensitized guinea pigs have a strong, and often mortal, reaction to the initial provocative challenge, approximately half are completely desensitized by the time a second challenge is presented. The basis for the desensitization is unknown but has been reported previously (*2*, *5*). We did find that when the concentration of the albumin solution was reduced from 800 to 300 mg/100 ml during the first provocative challenge, none of the 17 treated animals died. Subsequent challenges by a solution of 800 mg/100 ml have invariably been well tolerated. All animals that exhibited allergic attacks to a second provocative challenge continued to do so to subsequent challenges by the protein.
10. Plethysmographic measurements were kept short because accumulation of expired CO_2 in the closed chamber might have led to an artifactual constriction of the airway [G. N. Loofbourrow, W. B. Wood, I. L. Baird, *Amer. J. Physiol.* **191**, 411 (1957)].
11. Both UR's and CR's occurred as essentially all-or-none rises in plethysmographic amplitude, the actual increment being three- to fourfold for most animals. The absence of graded levels may have been due to the relatively lengthy, 3-minute presentations of CS's and US's and to the testing of UR's only after blocks of five trials had been conducted; however, even "on-line" plethysmographic recordings taken from human asthmatic patients exhibit the same all-or-none changes of pressure (*16*).
12. A short-lived experiment involved bilateral section of the vagus nerve in four guinea pigs and unilateral section in two controls. Sections were made in the neck a few millimeters caudad to the lower mandible. None of the four experimental animals recovered from anesthesia; both controls did. The guinea pig's apparent critical need for a unilaterally intact vagus prevented us from learning whether its fibers participate, as we suspect, in the conditional attack.
13. A sensitized animal reacting to provocative challenges may concomitantly develop a conditional attack; to control for such a dual occurrence, when pharmacologically treating an allergic attack we always first presented sham challenges to ensure absence of a CR. To control for the possibility that the CR had extinguished, when pharmacologically treating a conditional attack, we always presented a second sham challenge 1 to 3 days later, after dissipation of a drug, to insure the existence of the CR.
14. We refer to L. S. Goodman and A. Gilman, Eds., *The Pharmacological Basis of Therapeutics* (Macmillan, New York, ed. 3, 1965), sect. 4, chap. 21–28; *ibid.*, sect. 5, chaps. 29 and 30.
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17. These data were originally presented at the Annual Meeting of the American Psychological Association in San Francisco on 1 September 1968. The paper is based in part upon thesis research performed by E.W.B. for the Kansas University School of Medicine and was supported by 8200 Research Funds from the Veterans Administration and by a grant from the Kansas University Medical Center. We thank B. Ascough, E. B. Brown, D. C. Greaves, G. N. Loofbourrow, E. J. Walaszek, and E. L. Wike for technical or material assistance. We thank Prof. H. Lal for helping us select and evaluate effects of drug treatments.

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Glue Sniffing Causes Heart Block in Mice

Abstract. *In mice, the inhalation of airplane glue or toluene fumes slows the sinoatrial rate, prolongs the P-R interval, and sensitizes the heart to asphyxia-induced atrioventricular block. In humans who sniff glue or solvents, similar mechanisms may be a cause of sudden death.*

Bass reported on 110 cases of sudden death in youths turning on by inhaling the vapors of airplane glue, aerosol propellants, and various solvents (*1*). Although the fatal mechanism is unknown, the combination of rapid death and negative autopsy findings suggests that a cardiac arrhythmia might be responsible. To test this possibility, we investigated the electrocardiographic effects of glue sniffing in mice.