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Report

Antiperspirant Activity of H_1-Histamine Blockers as Determined by a Modified Rat Foot Pad Assay

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A method for inducing sweating in the rat via heat stress and without the use of general anesthetics is presented. Five commonly used H_1-blocking antihistamines were evaluated in this model for their antiperspirant efficacy. The antihistamines evaluated and their ED_{50} values (μg base/pad) were as follows: phenindamine, 3.02; diphenhydramine, 3.25; chlorpheniramine, 3.12; tripelennamine, 4.91; and pyrilamine, 13.03. Atropine sulfate, injected into the foot pads, was also found to inhibit the sweat response. The response to atropine varied directly with dose. The ED_{50} was estimated to be 0.4 ng base/foot pad. No systemic effects or contralateral involvement were seen. The rat foot pad contains eccrine sweat glands that are innervated by sympathetic cholinergic fibers. This relationship is analogous to that in the eccrine sweat glands of man. The rat data suggest that antihistamines, possibly via an anticholinergic effect, may be useful as potential antiperspirants in man.

KEY WORDS: H_1 blockers; anticholinergic; antiperspirancy; rat food pad.

INTRODUCTION

Acknowledgment of the presence and histological characterization of eccrine sweat glands was first made 40 years ago (1). It is now accepted that rat eccrine sweat glands, like eccrine sweat glands in man, are innervated by sympathetic cholinergic fibers.

Ring and Randall (1) demonstrated that sciatic nerve stimulation activated the rat food pad sweat glands. Increased basal body temperature had no effect in their studies. More recently, it has been shown that the systemic or local administration of cholinomimetic drugs such as pilocarpine (2–4) and mecholyl (5) or acetylcholine (4) stimulated sweating. Similarly, in man, the intradermal injection of cholinergic materials provoked the sweat response (6).

Several investigators have shown that anticholinergic drugs block the sweat response. It has been demonstrated in the rat foot pad that, with local injection, atropine blocks the response provoked by acetylcholine, mecholyl, or pilocarpine (4). Lansdown (3) demonstrated that atropine (subcutaneous, cervical region) blocks pilocarpine-induced rat food pad sweating.

Goodall reported that several topically applied H_1 antihistamines were able to inhibit acetylcholine-induced sweat production on the human forearm (8). Additionally, it has been demonstrated that anticholinergic materials given systemically, topically, or by intradermal injection to man can inhibit eccrine sweating (6–8).

It is generally accepted that most H_1-blocking antihistamines possess anticholinergic activity (9). Studies of the eccrine sweat response in man and rat indicate that antiperspirancy can be achieved by anticholinergic intervention. This use of antihistamines has not been systematically evaluated.

We have improved the Lansdown (3) rat foot pad model for testing antiperspirancy and used it to evaluate the antiperspirant potency of antihistamines. Inhibition of heat-evoked sweat was used as the efficacy end point.

MATERIALS AND METHODS

Animals

Male and female Sprague–Dawley [Tac: N(SD) f BR] rats weighing between 175 and 250 g were selected from a stock colony. All animals were acclimated to laboratory conditions for at least 7 days prior to use. Animal husbandry was as outlined in the "Guide for the Care and Use of Laboratory Animals" (10).

Rat Foot Pad Antiperspirancy: Animal Model

The method outlined by Lansdown (3) was modified to allow for the use of conscious rats, injection directly into the foot pad, and sweat induction by heat stress. Groups of at least five rats were used. Each animal received Innovar-Vet (0.04% fentanyl and 2.0% droperidol), 0.01 ml/100 g body weight intramuscularly, as a tranquilizer and analgesic. After approximately 10 min, each animal was suspended in a harness to prevent contact between the feet and any surface.

The harness was made using a 15 × 20-cm rectangular sheet of polypropylene. Holes were cut to allow the rat leg's

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to be drawn through (Fig. 1). The sheet was folded around the animal and held together with binder clips from which the harness was suspended. Both hind feet were swabbed with 70% ethyl alcohol to remove debris. Each of the four interdigital palmar foot pads on one foot was injected subcutaneously (0.01 ml/pad) with the appropriate test material using a 27-G needle. The four interdigital pads of the opposite (control) foot were each injected with 0.01 ml of distilled water. Both hind feet were swabbed with an iodine solution (2% w/v in 95% ethanol). The animal was left suspended and undisturbed. After 30 min, the ambient room temperature was elevated to 78 ± 2°F. Both hind feet were painted with a suspension of starch in castor oil, 50% (w/v). Ten minutes after application, Polaroid photos (3×; using Polaroid Type Film 669) were taken of the feet.

Active sweat glands appeared as black dots, formed as the sweat solubilizes the iodine, which is in turn trapped in the starch oil mixture (11). Using the photographs, dots on each pad were counted and added to obtain a total for each foot. Percentage inhibition was calculated as

$$\left[1 - \frac{\text{(number sweat glands/treated foot)}}{\text{(number sweat glands/control foot)}}\right] \times 100 = \% \text{ inhibition}$$

Drugs

Atropine sulfate, pyrilamine maleate, diphenhydramine HCl, and tripelennamine HCl were obtained from Sigma. Phenindamine tartrate was purchased from Roche, and chlorpheniramine maleate from Loftus Bryan. All test materials were freshly prepared utilizing house distilled water. Atropine sulfate was tested at doses of 0.03, 0.3, 3.0, and 30.0 ng/pad. Each of the antihistamine salts was tested at doses of 3, 16, and 30 μg/pad.

Statistical Analysis

ED_{50}'s were calculated for each drug expressed as the base. The calculated response variable was the ratio of active sweat glands on the treated paw to active sweat glands on the water-treated paw. Linear regression analysis methods implemented on SAS (12) were used to calculate the ED_{50}'s and their 95% confidence intervals for the antihistamines. Due to the nonlinear nature of the data, the ED_{50} for atropine was calculated with SAS using a quadratic model.

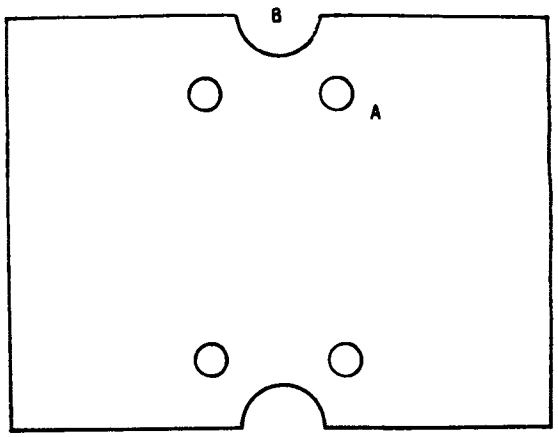

Fig. 1. Harness for rat foot pad studies. Rat legs are placed through holes (A). Head and tail are placed in notched areas (B). The harness is folded around the animal and held together with clips from which the harness is suspended. Harness dimensions: overall, 15 × 20 cm; distance between holes for contralateral legs, 5 cm; distance between holes for lateral legs, 10 cm.

RESULTS

Each drug was tested in salt form. Each salt contains a base which is responsible for antiperspirant activity; the amount of base present (as a percentage of molecular weight) is different for each drug. The corresponding amounts of base present at each dose were calculated and these data are provided in Table I.

Atropine produced dose-related inhibition of the sweat response. The data are summarized in Table I. Mean percentage inhibition ranged from 40 to 91% at the doses evaluated. The ED_{50} was estimated to be 0.4 ng base/pad. No signs of systemic toxicity were noted following the local injections of atropine sulfate.

The antiperspirant efficacy of each antihistamine also increased with ascending dose (Table I).

The highest mean response (92% inhibition) was observed from the high dose (26.1 μg base/pad) of diphenhydramine, followed closely by the high doses of chlorpheniramine and phenindamine, with 88 and 87% inhibition, respectively. The dose–response curves of percentage inhibition vs micrograms antihistamine base per pad are shown in Fig. 2.

Table I includes the ED_{50} for each antihistamine tested. Among the antihistamines phenindamine, diphenhydramine, and chlorpheniramine were the most potent antiperspirants, with similar ED_{50} values. Tripelennamine was slightly less effective. Pyrilamine was clearly the least potent. As expected, atropine sulfate was the most potent of all the drugs tested.

DISCUSSION

The eccrine sweat gland is innervated by sympathetic fibers which are predominantly cholinergic rather than adrenergic. This has been demonstrated pharmacologically in the rat (2–5) as well as in man (6–8). Recent studies, utilizing selective staining techniques and choline acetyltransferase measurements, have traced the development of sympathetic nerve fibers in neonatal rat foot pads. Initially the fibers are adrenergic but by day 21 of life they become cholinergic. This change roughly follows the development of the sweat gland, which begins shortly after birth and is largely complete by day 21 (12,13).

Sweat production in the rat foot pad can be induced by electrical stimulation of the sciatic nerve (1), systemic injection of mecholyl (5), systemic injection of pilocarpine (2,3), and injection of pilocarpine directly into the foot pad. Since the failure of Ring and Randall (1) to induce sweat by increasing body temperature, no reports have appeared in the literature with regard to sweat production induced by heat stress. Lansdown presented an elegant model for demonstrating the antiperspirant activity of topically applied materials (3). Using Lansdown's procedure, we have previously evaluated diphenhydramine HCl, an H_1 antihistamine, as an

Table I. Summary Table

Test material	Dose (μg salt/pad)	Calculated dose (μg base/pad)	Inhibition (%)	ED_{50} (μg base/pad)	95% confidence interval
Atropine	0.00003	0.000013	40	0.0004	—[a]
	0.0003	0.00013	50		
	0.003	0.0013	56		
	0.03	0.013	91		
Phenindamine	3	2.0	44		
	16	10.6	63	3.02	1.29, 4.76
	30	19.8	87		
Chlorpheniramine	3	2.1	32		
	16	11.2	81	3.12	1.80, 4.43
	30	21.0	88		
Diphenhydramine	3	2.6	47		
	16	13.9	75	3.25	1.72, 4.77
	30	26.1	92		
Tripelennamine	3	2.6	34		
	16	13.9	67	4.91	1.87, 8.08
	30	26.1	78		
Pyrilamine	3	2.1	25		
	16	11.4	36	13.03	4.67, —[a]
	30	21.3	62		

[a] Confidence limit could not be determined, due to either the slope or the nonlinear nature of dose–response curve.

antiperspirant (unreported results). In this model diphenhydramine inhibited pilocarpine-induced perspiration (approximately 40% inhibition).

The current paper describes several improvements to the Lansdown's procedure, increasing the practicality and relevance of the model. These modifications include the use of a harness and tranquilizer rather than general anesthesia, microinjection into the foot pads, and the use of a photographic record. Utilizing this improved method we have demonstrated that the rat foot pad will respond to environmental thermal stress. The harness system we described allows the use of tranquilizing doses of Innovar-Vet rather than the use of general anesthesia. General anesthesia may decrease body temperature, altering the sweat response. In addition, we have found that the combination of general anesthesia and pilocarpine is often toxic.

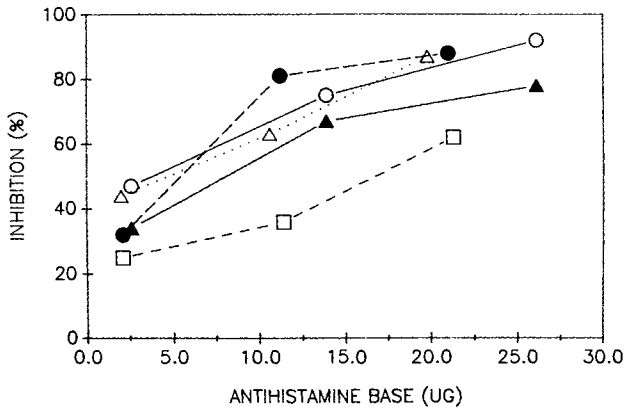

Fig. 2. Dose–response curves for antihistamine-induced sweat inhibition. Dose–response curves are depicted for inhibition of rat foot pad sweating by five antihistamine bases. Diphenhydramine (open circle), chlorpheniramine (filled circle), phenindamine (open triangle), tripelennamine (filled triangle), and pyrilamine (open square).

It is recognized that the fentanyl component of Innovar-Vet may produce cholinergic side effects. Cholinergic effects in this model, however, would tend to enhance the sweat response. Since Innovar-Vet is administered systemically, this effect would be bilateral, affecting both the control and the test pads.

The harness system allows for injection of materials directly into the foot pad. Anticholinergics are not active antiperspirants at the surface of the foot but must penetrate to the level of sweat gland innervation. If applied topically, penetration of these materials is dependent on concentration, vehicle, use of penetration enhancers, and thickness of the stratum corneum. Local injection dissociates the variables of efficacy and penetration and allows for direct comparison of activity at the level of the gland.

Finally, taking Polaroid photos allows us to count the active sweat glands after the exposure period. This makes the procedure more convenient and quantitative, as the actual number of active glands in each paw can be determined.

Goodall (8) reported that several antihistamines block eccrine sweat production in man. His work involved a subjective evaluation of sweat inhibition. In Goodall's study, occlusive topical application of the antihistamines was followed by intradermal injection of acetylcholine. The present study utilizes an objective evaluation of sweat inhibition without the direct application of a stimulant (acetylcholine or pilocarpine).

The study data demonstrate that the injection of atropine sulfate, a potent anticholinergic material, directly into the rat foot pad will block the thermal response of the sweat glands. Thirty nanograms of atropine sulfate (13 ng base) produced a nearly total block of the sweat response.

Likewise, antihistamines, when injected in microgram

quantities, were also effective antiperspirants. The antihistamines are weak anticholinergics, relative to the belladonna alkaloids, as is reflected by their higher ED_{50}'s. It is suggested that the antiperspirant properties of the antihistamines are the result of anticholinergic actions. The antihistamines are also known to produce a local anesthetic effect. Consideration was given to the possibility that the antiperspirancy seen in this study might be due to a nerve block, rather than an anticholinergic effect.

Injection studies utilizing 15 μg of proparacaine per pad (Opthaine, Squibb, Lot 417601) produced only 17% inhibition of sweat (unreported results). In the present study, injection of 16 μg of various antihistamine salts produced from 36% inhibition for the weaker to 75% inhibition for the most potent. Each of the antihistamine salts, at 3 μg/pad, produced more inhibition of sweat than did proparacaine at a dose of 15 μg/pad. These findings suggest that it is the anticholinergic property and not an anesthetic effect of the antihistamine which is responsible for antiperspirant efficacy.

Kubo *et al.* (15) studied the antimuscarinic affinity of a large series of H_1-blocking antihistamines via inhibition of binding to acetylcholine receptors in bovine cerebral cortex. The rank order of the inhibition constants was diphenhydramine, chlorpheniramine, and tripelennamine. This is the same rank order of antiperspirant ED_{50}'s found in the present research.

In conclusion, it appears from this study that the antihistamines can inhibit thermally induced sweat in a rat foot pad model. It is suggested that this effect is related to the anticholinergic activity of the antihistamines.

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