Quantitative Studies on Passive Cutaneous Anaphylaxis in the Denervated Skin of the Guinea Pig* (33046)

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Recent investigations have revealed a relationship between the central nervous system and systemic anaphylaxis. Several studies (1–3) have shown that various central nervous system (CNS) lesions in the guinea pig, rabbit, and rat can offer protection against lethal anaphylactic shock. It has also been shown that mice subjected to avoidance conditioning stress show decreased susceptibility to passive systemic anaphylaxis (4) and guinea pigs subjected to the stress of excessive handling may not give passive cutaneous anaphylactic (PCA) reactions (5). The effect of local skin denervation on PCA in the guinea pig was qualitatively studied (6) and it was shown that local denervation has no effect on PCA performed with rabbit antibodies in the guinea pig. The purpose of this study was to quantitatively investigate the relationship with both rabbit and guinea pig γG1 antibody fractions.

Materials and Methods. Oval patches of skin of female 300-gm Hartley strain albino guinea pigs were denervated according to a modified method of Mansfeld (6,7). The guinea pigs were shaved on one side and the remaining hair was removed with a depilatory. A flap of skin was raised and replaced using 9-mm autoclips (Clay Adams). Care was taken to ligate bleeding vessels. Seven to 10 days later another portion of skin was raised so that an entire oval patch of skin on one side of the guinea pig had been raised. After 7–10 days, PCA was performed as previously described (5). The unoperated side was used as a control. At this time, i.e., 7–10 days after the second operation, the skin of the operated sides was still slightly indurated and the animals did not respond to pinching of the skin on the operated site, but did on the opposite side.

Guinea pigs were immunized with highly derivatized dinitrophenylated guinea pig albumin (DNPGPA) and the separation of γG1 fractions were performed as described previously (8). Rabbits were immunized with highly derivatized dinitrophenylated bovine gamma globulin (DNPBGG) as previously described (9). Rabbit sera and guinea pig γG1 fractions were titrated for antibody protein content (Ab) by the classical microprecipitin method of Heidelberger and Kendall.

For the challenge of PCA reactions the antigen was prepared in the following manner. Bovine serum albumin (BSA) was coupled to 2,4-dinitrophenyl-sulfonic acid as previously described (10). There were 28 dinitrophenyl (DNP) groups per molecule of BSA ($DNP_{28}BSA$). The antibody dilutions were injected intradermally symmetrically into the operated and unoperated side. 0.1-ml rabbit anti-DNPBGG serum (400 μg of Ab/ml) in dilutions of 1:1000 and 1:2000 were injected into six guinea pigs and the animals were challenged intravenously with 325 μg $DNP_{28}BSA$ and 1 ml 0.5% Evans blue 4 hours later. Three animals were injected with 0.1 ml of guinea pig γG1 fraction anti-DNPGPA antibodies (800 μg of Ab/ml) in dilutions of 1:1000, 2000, and 4000. The challenge procedure was similar. The animals were killed 30 min after challenge. The reactions were read as the largest diameter in millimeters 20 min after challenge on the internal side of the skin.

Results and Discussion. In all of the animals, the threshold level of the reaction appeared to be within one dilution for the operated and unoperated sides (see Table I). The slight induration of the skin of the operated side may have tended to decrease the degree of reaction and it was felt that there were no significant differences between the

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TABLE I. The PCA Reactions with Rabbit and Guinea Pig γG1 Antibodies in Denervated and Intact Skin of the Guinea Pig.

Antibody and location (side)	Ab (μg/ml)	Guinea pig no.:	Diameter of PCA reactions (mm) 1	2	3	4	5	6
A. Rabbit								
Denervated	0.40		12	10	14	10	9	7
	0.20		0	0	0	0	0	0
Unoperated	0.40		12	15	14	14	10	12
	0.20		tr	6	10	10	tr	0
		Guinea pig no.:	7	8[a]	9			
B. Guinea pig γG1								
Denervated	0.80		15	10	12			
	0.40		8	6	10			
	0.20		6	0	tr			
Unoperated	0.80		13	15	15			
	0.40		10	12	10			
	0.20		8	6	tr			

[a] The denervated side of the skin of this animal was more indurated than the denervated side of the skin of the other animals.

degree of the PCA reactions at the denervated or unaltered sites.

The mechanism by which the nervous system effects the anaphylactic reactions is unknown. The role of neuroendocrine processes, sympathetic–parasympathetic mechanisms, and histamine release have been considered. Changes in rate of antibody synthesis are not essential as effects are seen with CNS lesions in passive transfer experiments (1). Using a localized anaphylactic reaction it is possible to study the effects of removal of one of the denominators of the nervous system, the peripheral neuron. Peripheral nervous system does not seem to play any role in the mechanism of sensitization by either heterologous (rabbit) or homologous (guinea pig γG1) antibodies.

Anaphylactic reactions involve many steps such as the "fixation" of antibodies to tissue receptors (5), the interaction of antibody with antigen, the liberation of vasoactive substances, and finally the action of the latter on the permeability of small venules. As the reactions were not inhibited, none of the steps were influenced by the denervation.

Summary. Denervation of the skin of guinea pigs does not inhibit passive cutaneous anaphylactic reactions with either rabbit γG or guinea pig γG1 antibodies.

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