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PHOTOAFFINITY LABELING OF H_1-HISTAMINE RECEPTORS IN ISOLATED SMOOTH MUSCLES BY 4(5)-[2-(4-AZIDO-2-NITROANILINO)ETHYL]IMIDAZOLE: CHARACTERIZATION IN GUINEA-PIG AORTA AND LACK OF PHARMACOLOGICAL ANTAGONISM IN DOG TRACHEALIS *

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The compound 4(5)-[2-(4-azido-2-nitroanilino)ethyl]imidazole (AAH), a photoaffinity label analog of histamine, has been observed previously to produce a photolysis-dependent, specific and irreversible antagonism of histamine-induced contractile responses of the guinea-pig vas deferens. The pharmacological effects of AAH in isolated guinea-pig aorta (GPA) and dog trachealis (DT) were evaluated in the present study. Photolysis of 3×10^{-5} M AAH for 5 min with visible light in organ baths containing the GPA resulted, after washout of AAH, in a nonequilibrium competitive antagonism of contractile responses to histamine; responses to norepinephrine and KCl were not affected. In contrast, photolyzed AAH (3×10^{-5} M, one or two photolysis treatments; 10^{-4} M AAH, two treatments) did not antagonize contractile responses of the DT to histamine, nor were responses to acetylcholine or KCl affected. To test the possibility that AAH lacks affinity for histamine receptors in the DT, molar K_B values were obtained for nonphotolyzed AAH used as a conventional equilibrium competitive antagonist; K_B values for GPA and DT were not different ($-\log K_B$ were 5.48 ± 0.18 and 5.04 ± 0.23 for GPA and DT, respectively; $P>0.05$). pA_2 values for diphenhydramine, an H_1-histamine receptor antagonist, indicated a moderately greater potency in GPA compared to DT (pA_2 were 7.58 ± 0.10 and 6.89 ± 0.13 for GPA and DT, respectively; $P<0.05$); however the diphenhydramine:AAH potency ratio did not explain the resistance of the DT to photolyzed AAH. Cimetidine ($10^{-6}-10^{-4}$ M), an H_2-receptor antagonist, potentiated contractile responses of the GPA to histamine but was without an effect in the DT. No evidence for relaxation by histamine in preparations with induced tone was obtained; the resistance of the DT to photolyzed AAH therefore does not involve an inhibition of H_2-inhibitory receptors. Photolyzed AAH thus demonstrates tissue-selective antihistamine activity, perhaps as a result of differences in the characteristics of H_1-receptors in the GPA and DT.

H_1-histamine receptor Antagonist Photoaffinity label Guinea-pig aorta Dog trachealis

1. Introduction

A photoaffinity analog of histamine, 4(5)-[2-(4-azido-2-nitroanilino)ethyl]imidazole, or AAH, was synthesized and tested for pharmacological activity in our laboratory. Photolysis of AAH with visible light in organ baths containing the guinea-pig vas deferens, following washout of the compound, resulted in a specific and irreversible antagonism of contractile responses to histamine (O'Donnell et al., 1981). If the AAH was not photolyzed, no antagonism was seen after its washout; when present in the organ bath during

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histamine additions, nonphotolyzed AAH antagonized the responses. Photolysis of AAH in the presence of diphenhydramine, an H_1-receptor antagonist, resulted in protection of the tissue from the effect of AAH, while histamine provided no protection and cimetidine, an H_2-receptor antagonist, provided weak protection. The results suggested that AAH, upon photolysis, became covalently attached to an H_1-receptor antagonist binding site.

The affinity of AAH for the histamine receptor in the guinea-pig vas deferens could not be estimated because histamine concentration-response curves were 'bell'-shaped in the presence of nonphotolyzed AAH or after photolysis of AAH. Moreover, all excitatory agonists cause phasic contractile responses in this tissue. Whether agonists in the biophase are at equilibrium with receptors throughout the tissue during the peak of contraction, an assumption which must be made for kinetic analysis, is somewhat uncertain because there is considerable electrical coupling in the vas deferens (Goto et al., 1977).

The present studies were conducted to evaluate the effects of AAH in the smooth muscle of the guinea-pig aorta and dog trachealis. These tissues develop well-maintained tonic contractions which are more amenable to kinetic analysis under equilibrium conditions. They were selected so as to obtain a comparison between two tissues of the guinea pig and an unrelated muscle from the dog.

2. Materials and methods

2.1. Preparation of tissues

Adult, English short-hair male guinea pigs (250–350 g; Hilltop Lab Animals, Scottdale, PA) were killed by a blow to the head and bled. The aorta was removed, placed in modified Krebs-Henseleit solution (O'Donnell et al., 1981) and dissected free of adventitia. Spiral strips (15°; 2 mm × 1.5 cm) were mounted and attached to a transducer for the measurement of isometric contractile responses. Each preparation was placed in a separate 20 ml glass, water-jacketed organ chamber containing modified Krebs-Henseleit solution (37°C); resting tension was 400 mg.

Mongrel dogs of either sex (14–21 kg) were anesthetized with sodium pentobarbital (40 mg/kg i.v.) and the trachea was removed. The trachealis muscle from individual ring segments, beginning with the sixth ring from the larynx and extending posteriorly, was cleared and divided, in a direction parallel to the fiber bundles, into two paired strips (1.5–2 mm in width by 5–6 mm in length). The strips were mounted and placed in organ chambers for the measurement of isometric tension as described for the GPA (vide supra). Resting tension was 1 g.

2.2. Concentration-response studies

Tissues were incubated in Krebs-Henseleit solution for 1 h prior to the experiment; the solution was changed every 15 min. Agonist agents were added to the bath with cumulative increases in concentration. Where appropriate, diphenhydramine or cimetidine were incubated with the tissue for 30 min prior to the addition of agonist and remained present thereafter. Each tissue was used to develop only one concentration-response curve.

Drug effects on agonist concentration-response curves were quantitated by obtaining dose ratios (e.g., EC_{50} in the presence of the agent or after photolysis of AAH divided by EC_{50} for control, where EC_{50} is the effective molar agonist concentration which results in 50% of the maximum response). K_B and pA_2 values were calculated from dose ratios (Arunlakshana and Schild, 1959).

2.3. Experiments with 4(5)-[2-(4-azido-2-nitroanilino)ethyl]imidazole (AAH)

The synthesis of AAH has been reported previously (O'Donnell et al., 1981). In the present study the effects of AAH were studied using the same protocols as described in O'Donnell et al. (1981), except as noted in results. Briefly, these were as follows.

2.3.1. Effect of AAH after photolysis

Test tissues in the organ bath were incubated with AAH for 5 min. The preparations were then

irradiated with a tungsten-halogen projector lamp (DVY, 650 W, 3400°K, bulb filament ca. 10 cm from the tissue) for 5 min. At the end of irradiation the tissues were washed twice, at 5 min intervals, with fresh Krebs-Henseleit solution to remove the AAH. Control tissues were irradiated for 5 min in the absence of AAH. Concentration-response determinations were begun 10 min after the end of the irradiation period.

2.3.2. Effects of nonphotolyzed AAH

To examine the effect of transient incubation of tissues with nonphotolyzed AAH, test tissues were exposed to AAH for 15 min without irradiation. The tissues were washed twice, as above, before agonists were added. Control tissues were washed but otherwise received no treatment.

Responses to agonists in the presence of nonphotolyzed AAH were also evaluated. Tissues were incubated in nonphotolyzed AAH for 15 min prior to, and during, concentration-response determinations. Control tissues received no treatment.

2.4. Chemicals

Histamine diphosphate, acetylcholine bromide and norepinephrine bitartrate were from Sigma Chemical Co. Diphenhydramine hydrochloride was from Parke, Davis and Co. Cimetidine was from Smith, Kline and French. All other chemicals were the highest grades available.

2.5. Statistical analysis

The data were evaluated for differences with Student's t-test for paired or unpaired samples, as appropriate. A probability less than 0.05 was considered significant. Slopes of Schild plots were determined using linear regression. Means are presented ±S.E.M. EC_{50} values were obtained from regression analysis of probit-transformed data and are presented as $-\log EC_{50}$. n is the number of separate experiments.

3. Results

3.1. Effects of AAH in guinea-pig aorta

AAH (3×10^{-5} M) did not cause contraction when added to the organ bath. Photolyzed AAH (3×10^{-5} M) caused an 11.5-fold shift of the histamine concentration-response curve to the right of the control and a 38% reduction in maximum response (fig. 1 and table 1(A)). When normalized in terms of the tissue's maximum response (fig. 1, right-hand panel), the concentration-response curve of AAH-treated tissues was shifted to the right in a parallel fashion.

The concentration-dependence of the effect of photolyzed AAH was examined. Photolysis of 10^{-5} M AAH reduced the maximum response to histamine by 19% but had no effect on the EC_{50} of histamine (table 1(B)).

To determine the specificity of antagonism by photolyzed AAH, the effects of AAH on concentration-response relationships of chemically-unrelated agonists was examined. The results are summarized in table 1. Photolysis of 3×10^{-5} M and 10^{-5} M AAH had no effect on the EC_{50} values or maximum responses of the tissues to norepinephrine and KCl. The ability of AAH to produce a weak muscarinic receptor antagonism,

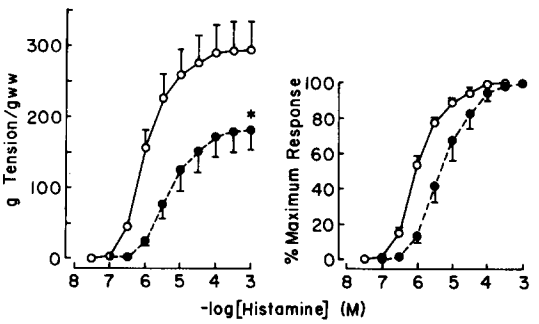

Fig. 1. Histamine concentration-response curves for guinea-pig aorta following photolysis ('+hν'; 5 min) and washout of 3×10^{-5} M AAH. *Left panel*: contractile tension responses normalized in terms of grams wet weight (gww) of tissue. *Right panel*: data from the left panel expressed as percentage of the tissue's maximum response to histamine. Values for EC_{50} and maximum response are summarized in table 1A. * Significantly less than control. ○ Control (+hν); ● 3×10^{-5} M AAH (+hν).

TABLE 1

Effect of AAH on concentration-response relationships of guinea-pig aorta.

	Histamine		Norepinephrine		KCl	
	$-\log EC_{50}$	Maximum response [a]	$-\log EC_{50}$	Maximum response	$-\log EC_{50}$	Maximum response
(A) 3×10^{-5} M AAH (+hν)						
Control	5.82±0.09	293±41	5.83±0.12	165±35	1.46±0.03	95±19
AAH	5.19±0.18 [c]	182±26 [c]	5.51±0.10	146±22	1.43±0.05	128±24
DR [b]	11.52±8.46 (6)		2.93±1.15 (6)		1.11±0.10 (6)	
(B) 10^{-5} M AAH (+hν)						
Control	5.80±0.11	306±24	5.81±0.08	212±17	1.54±0.05	249±43
AAH	5.67±0.13	248±38 [c]	5.69±0.04	213±24	1.53±0.04	212±31
DR	1.51±0.35 (5)		1.43±0.23 (8)		1.04±0.12 (5)	
(C) 3×10^{-5} M AAH (−hν; washout)						
Control	5.85±0.17	333±48				
AAH	5.71±0.12	287±44				
DR	1.53±0.33 (4)					
(D) 10^{-5} M AAH (−hν; washout)						
Control	5.96±0.20	349±25				
AAH	6.14±0.22	316±21				
DR	0.70±0.13 (4)					
(E) 3×10^{-5} M AAH (−hν)						
Control	5.74±0.16	197±28	6.06±0.08	239±30	1.52±0.04	254±29
AAH	4.72±0.07 [c]	228±39	5.92±0.06	267±42	1.36±0.04 [c]	146±26 [c]
DR	14.58±5.14 (6)		1.49±0.30 (5)		1.50±0.17 (6)	
(F) 10^{-5} M AAH (−hν)						
Control	5.96±0.20	337±20				
AAH	5.75±0.24 [c]	271±42				
DR	1.65±0.16 (4)					

[a] g tension/gww. [b] Dose ratio: (EC_{50} AAH)/(EC_{50} Control). [c] $P<0.05$ compared to control. n in parentheses. '+hν': AAH photolyzed in presence of tissue and washed out before agonists were added. '−hν; washout': AAH incubated for 15 min but washed out prior to adding agonist. '−hν': AAH incubated for 15 min before and during addition of agonist.

as occurs in guinea-pig vas deferens (see O'Donnell et al., 1981) was not possible because acetylcholine (10^{-9}–10^{-2} M) did not contract the tissue (n = 3).

Experiments were performed to determine if the antagonism by photolyzed AAH was photolysis-dependent and not a result of incubation of the tissues with the compound per se. The results are summarized in table 1 (C and D). Incubation of the tissues with 3×10^{-5} or 10^{-5} M AAH for 15 min, the total exposure time used when the AAH was photolyzed, had no effect on the EC_{50} values and maximum responses of the tissues to histamine. That is, AAH produced no histamine antagonism following its washout unless the compound was photolyzed in the presence of the tissues. The antagonism by photolyzed AAH was not the result of a spontaneous chemical change or a metabolic activation by the tissue.

It is appropriate to demonstrate that AAH has an affinity for the histamine receptor prior to the formation of covalent bonds upon photolysis (O'Donnell et al., 1981). Concentration-response curves for histamine were therefore obtained in the presence of nonphotolyzed AAH. Fig. 2 and table 1 (E and F) show that histamine concentration-response curves were shifted to the right of control in a parallel fashion in the presence of nonirradia-

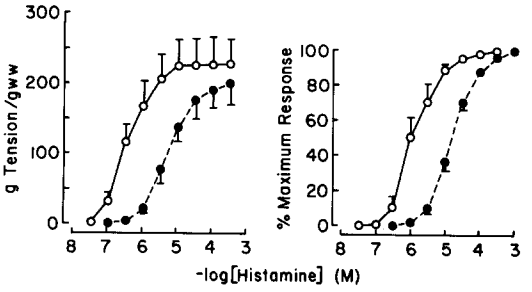

Fig. 2. Histamine concentration-response curves for guinea-pig aorta obtained in the absence and presence of nonphotolyzed ('$-h\nu$') 3×10^{-5} M AAH. Values for EC_{50} and maximum response are summarized in table 1C. ○ Control ($-h\nu$); ● 3×10^{-5} M AAH ($-h\nu$).

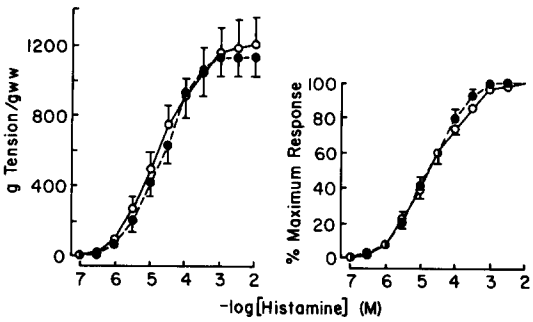

Fig. 3. Histamine concentration-response curves for dog trachealis following photolysis ('$+h\nu$'; 5 min) and washout of 3×10^{-5} M AAH. Values for EC_{50} and maximum response are summarized in table 2A. ○ Control ($+h\nu\times1$); ● 3×10^{-5} M AAH ($+h\nu\times1$).

ted 3×10^{-5} M and 10^{-5} M AAH; the compound had no effect on the maximum response to histamine under these conditions, i.e., the antagonism showed equilibrium competitive kinetics.

It was of interest to evaluate the specificity of the antagonism in the presence of nonphotolyzed AAH. While there was no effect on norepinephrine concentration-response curves, the EC_{50} of KCl was increased and the maximum response was significantly decreased (table 1). This latter finding was not observed in parallel studies on dog trachealis (vide infra) or in the previous study on guinea-pig vas deferens.

3.2. Effects of AAH in dog trachealis

Fig. 3 and table 2(A) show that 3×10^{-5} M AAH, following one treatment involving photolysis, had no effect on the EC_{50} or maximum response of histamine concentration-response curves. Likewise, acetylcholine and KCl concentration-response curves were unaffected by photolyzed AAH.

The degree of antagonism produced by a non-equilibrium competitive antagonist is a function of both the concentration used and the duration of tissue exposure to the agent. More rigorous treatments with photolyzed AAH, i.e., increased contact time of tissue with photoconverting AAH and increased AAH concentration, were therefore used in an attempt to produce an antagonism. In two series of experiments the tissues were photolyzed in the presence of 3×10^{-5} or 10^{-4} M AAH two times: following the first photolysis period the tissues were washed to remove the AAH; 3×10^{-5} or 10^{-4} M AAH was immediately readded to the bath and, following a 10 min incubation, the preparations were photolyzed again for 5 min. The results of these experiments are summarized in table 2. Neither 3×10^{-5} M AAH (table 2(B)) nor 10^{-4} M AAH (table 2(C)) antagonized responses to histamine under these conditions. In fact, double exposures of the tissues to photolyzed AAH produced a small potentiation of responses, which was significant for 3×10^{-5} M AAH. To verify whether 3×10^{-5} M AAH photolyzed twice could evoke nonspecific pharmacological effects, concentration-response relationships for acetylcholine and KCl were obtained (table 2(B)). The EC_{50} and maximum responses of these agonists were not modified.

As observed in guinea-pig aorta, incubation of the preparations with nonphotolyzed 3×10^{-5} M AAH for 15 min had no effect on the histamine concentration-response curve (table 2(D)).

Inasmuch as photolyzed AAH failed to antagonize histamine-induced responses, it was possible that histamine receptors of the dog trachealis have a lesser affinity for AAH than those of the guinea-pig aorta. Concentration-response curves were therefore obtained in the presence of nonphotolyzed 3×10^{-5} M AAH, and the results of these experiments are shown in fig. 4 and table 2. AAH caused a 5.83-fold shift of the histamine concentration-response curve to the right of control

TABLE 2

Effects of AAH on concentration-response relationships of dog trachealis.

	Histamine		Acetylcholine		KCl	
	$-\log EC_{50}$	Maximum response [a]	$-\log EC_{50}$	Maximum response	$-\log EC_{50}$	Maximum response
(A) 3×10^{-5} M AAH (+hν×1)						
Control	4.59±0.11	1202±153	5.49±0.26	2652±210	1.62±0.02	1995±133
AAH	4.79±0.13	1136±115	5.37±0.19	2489±272	1.56±0.03	1778±174
DR [b]	1.01±0.26 (6)		3.10±2.36 (5)		1.16±0.09 (8)	
(B) 3×10^{-5} M AAH (+hν×2)						
Control	3.90±0.15	848±240	5.63±0.12	2057±320	1.53±0.04	1569±349
AAH	4.16±0.12 [c]	690±196	5.31±0.48	1629±183	1.57±0.05	1090±328
DR	0.54±0.11 (3)		2.06±0.83 (3)		0.91±0.04 (3)	
(C) 10^{-4} M AAH (+hν×2)						
Control	3.74±0.10	758±196				
AAH	4.18±0.12	794±104				
DR	0.44±0.16 (3)					
(D) 3×10^{-5} M AAH (−hν; washout)						
Control	4.63±0.08	1303± 95				
AAH	4.93±0.16	1577±165				
DR	0.60±0.04 (7)					
(E) 3×10^{-5} M AAH (−hν)						
Control	4.65±0.09	1276±115	5.48±0.16	3115±258	1.52±0.05	1884±206
AAH	3.98±0.10 [c]	847± 92 [c]	5.07±0.18	2853±462	1.48±0.06	1678±129
DR	5.83±1.12 (4)		3.46±1.77 (4)		1.08±0.48 (4)	

[a] g tension/gww. [b] Dose ratio: (EC_{50} AAH)/(EC_{50} Control). [c] $P<0.05$ compared to control. n in parentheses. '(+hν×1)': one treatment with photolyzed AAH; '(+hν×2)': two treatments with photolyzed AAH. See legend of table 1 for explanation of '(−hν; washout)' and '(−hν)'.

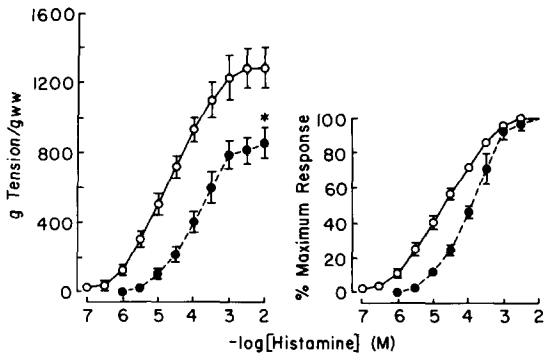

Fig. 4. Histamine concentration-response curves for dog trachealis obtained in the absence and presence of nonphotolyzed ('−hν') 3×10^{-5} M AAH. Values for EC_{50} and maximum response are summarized in table 2C. ○ Control (−hν); ● 3×10^{-5} M AAH (−hν).

(table 2(E)). For reasons unclear at present, nonphotolyzed AAH also reduced the maximum response to histamine by 34%. Notwithstanding the reduced maximum response, the rightward shift was roughly parallel. Concentration-response curves for acetylcholine and KCl were not affected in the presence of nonphotolyzed 3×10^{-5} M AAH (table 2(E)). Thus, the antagonism observed in the presence of nonphotolyzed AAH was specific for histamine in the dog trachealis. This is to be compared with the guinea-pig aorta (vide supra) in which nonphotolyzed AAH antagonized KCl-induced contractions.

3.3. Affinities of guinea-pig aorta and dog trachealis for nonphotolyzed AAH and diphenhydramine

Affinities of nonphotolyzed AAH for histamine receptors of guinea-pig aorta and dog trachealis

were compared. In these experiments, nonphotolyzed AAH (3×10^{-5} M) remained present during histamine additions. Affinities were given by K_B where $K_B = [AAH]/(\text{dose ratio} - 1)$. The results are summarized in table 3. There was no significant difference between the K_B values obtained for both preparations. If the lack of significant difference was ignored, AAH appeared ca. 2.8-fold more potent in guinea-pig aorta compared to dog trachealis. This calculation prompted a more complete investigation of possible differences in histamine receptors of the two tissues using diphenhydramine, a well-characterized H_1-receptor antagonist. pA_2 values were obtained using 10^{-7} and 3×10^{-7} M diphenhydramine in aorta and 3×10^{-7} and 10^{-6} M diphenhydramine in trachealis. In both tissues the slopes of Schild plots were not significantly different from unity (1.02 ± 0.41 and 1.05 ± 0.31 for aorta and trachealis, respectively). Table 3 shows that diphenhydramine was significantly, ca. 4.9-fold, more potent in guinea-pig aorta compared to dog trachealis. These results suggest that the histamine receptor of guinea-pig aorta has a greater affinity for both antagonists compared to dog trachealis. However, for both antagonists the relative affinities *between tissues,* namely 2.75 and 4.90 for AAH and diphenhydramine, respectively, were comparable. That is, this comparison provided no explanation for the greater antagonistic effect of AAH in guinea-pig aorta. Receptors may be classified based on relative potency series for agonist or antagonist binding. Using this approach, diphenhydramine was ca. 126-times more potent than AAH in aorta, and ca. 71-times more potent in trachealis (table 3). While this difference in relative potency may be regarded, tentatively, as meaningful, it is not as striking as the total lack of antagonist effect of photolyzed AAH in the dog trachealis.

3.4. Effects of cimetidine in guinea-pig aorta and dog trachealis

The small shifts of dog trachealis histamine concentration-response curves to the left of control produced by double photolysis treatments (table 2C and D) suggested that photolyzed AAH could be binding to inhibitory H_2-receptors, thereby masking the effect of blockade of excitatory H_1-receptors. Therefore, an indirect approach was first used to determine if the contractile response to histamine in dog trachealis and guinea-pig aorta could be modified by a conventional H_2-receptor antagonist, cimetidine. The results are shown in table 4. In the guinea-pig aorta, cimetidine produced a concentration-dependent shift of the histamine concentration-response curve to the left of control. The EC_{50} of histamine in the dog trachealis was not affected significantly by cimetidine.

The indirect evidence suggesting the presence of H_2-receptors in guinea-pig aorta, and their absence in dog trachealis, was studied more directly as follows. Test tissues were incubated for 30 min in the presence of 10^{-6} M diphenhydramine. Following the induction of tone (using 10^{-6} M norepinephrine in the aorta and 10^{-7} M methacholine in the trachealis), histamine was added to determine if relaxation occurs in the presence of H_1-receptor blockade. In the guinea-pig aorta histamine did not cause relaxation whether or not diphenhydramine was present; in the dog trachea-

TABLE 3

Affinities of nonphotolyzed AAH and diphenhydramine in guinea-pig aorta and dog trachealis.

	Guinea pig aorta	Dog trachealis	$\frac{K_B \text{ (trachealis)}}{K_B \text{ (aorta)}}$
AAH ($-\log K_B$; M)	5.48 ± 0.18 (6)	5.04 ± 0.23 [a] (4)	2.75
Diphenhydramine (pA_2; M)	7.58 ± 0.10 (4)	6.89 ± 0.13 [b] (4)	4.90
$\frac{K_B \text{ (diphenhydramine)}}{K_B \text{ (AAH)}}$ [c]	125.9	70.8	

[a] $P > 0.05$ compared to aorta. [b] $P < 0.05$ compared to aorta. [c] $pA_2 = -\log K_B$. n in parentheses.

TABLE 4

Effect of cimetidine on histamine concentration-response relationships of guinea-pig aorta and dog trachealis.

	$-\log EC_{50}$ (M)	DR [a]	Leftward shift (-fold) [b]
Guinea-pig aorta			
Control (4)	6.04 ± 0.07		
Cimetidine (M) (4)			
10^{-7}	6.21 ± 0.11	0.88 ± 0.41	1.73 ± 0.43
10^{-6}	6.42 ± 0.12 [c]	0.48 ± 0.14	2.81 ± 0.81
10^{-5}	6.44 ± 0.13 [c]	0.47 ± 0.14	2.88 ± 0.85
10^{-4}	6.44 ± 0.19 [c]	0.48 ± 0.16	3.04 ± 1.11
Dog trachealis			
Control (4)	5.02 ± 0.20		
Cimetidine (M) (4)			
10^{-6}	4.89 ± 0.15	3.67 ± 3.03	
10^{-5}	4.93 ± 0.08	2.39 ± 1.72	
10^{-4}	4.75 ± 0.04	2.38 ± 1.07	

[a] ($-\log EC_{50}$ cimetidine)/($-\log EC_{50}$ control). [b] ($-\log EC_{50}$ control)/($-\log EC_{50}$ cimetidine). [c] $P<0.05$. n in parentheses.

lis, small relaxations were occasionally produced by histamine, in the presence of diphenhydramine, but only in concentrations $\geq 10^{-2}$ M (n = 3 for each tissue; data not shown). These findings do not support the indirect evidence (table 4) for the presence of inhibitory H_2-receptors in the guinea-pig aorta, and they also confirm the indirect evidence (table 4) that H_2-receptors are not present to a significant extent in the dog trachealis. Thus, in neither tissue does an interaction between photolyzed AAH and H_2-receptors occur.

4. Discussion

Treatment of dog trachealis with photolyzed AAH, using conditions more rigorous than those which were effective in the aorta and vas deferens of the guinea pig, failed to antagonize histamine-induced contractions. A working hypothesis drawn from the present results is that *photolyzed* AAH distinguishes a difference in the H_1-receptors in the dog and guinea-pig tissues which were studied.

Several factors which could have contributed to the resistance of dog trachealis to photolyzed AAH were considered. (1) Light may not have penetrated the tissue with an intensity sufficient to activate subsurface AAH. This possibility is not likely for two reasons. The thickness of the preparations was not appreciably greater than the guinea-pig vas deferens in which the photolyzed compound is effective. High concentrations of AAH and two photolysis treatments should have generated a sufficient amount of nitrene intermediate to bind to the receptor. Such treatment, however, resulted in no antagonism. (2) AAH may have been metabolized or sequestered in the biophase. The lack of antagonistic effect of high concentrations of photolyzed AAH and two photolysis periods makes this possibility untenable. (3) The affinity of AAH for the H_1-receptor might be low. The K_B values for nonphotolyzed AAH in the guinea-pig aorta and dog trachealis were not significantly different. That is, the fractional occupancy of the receptors by 3×10^{-5} M AAH in both tissues, at the time when AAH is converted by photolysis from an equilibrium competitive antagonist into a nonequilibrium competitive antagonist, is the same. The resistance of the dog trachealis to photolyzed AAH is not, therefore, explainable on the basis of a lower affinity of nonphotolyzed AAH for the receptor than exists in the guinea-pig tissues.

A fourth possible explanation for the inability of photolyzed AAH to behave as an antagonist in dog trachealis is the simultaneous inhibition of both H_1- and H_2-receptors by the agent. No evidence was found for H_2-receptors in this tissue: cimetidine had no effect on the histamine concentration-response relationship (table 4) and histamine (10^{-7} to 3×10^{-3} M) did not cause relaxation of contracted preparations when diphenhydramine was present. We conclude that the inability of photolyzed AAH to antagonize histamine-induced contractions is not explainable on the basis of photoaffinity labeling of H_2-receptors. In addition, the small, leftward shift in the histamine concentration-response curve which resulted after two treatments with photolyzed AAH (table 2B and C), the observation which initiated the evaluation of H_2-receptors, is apparently not the result of H_2-receptor inhibition (see Chand and Altura, 1980).

A fifth possibility, which was not tested in the present study, is that photolyzed AAH (table 2A–C) but not nonphotolyzed AAH (table 2E) inhibits histamine catabolism in the dog trachealis to a greater extent than it blocks H_1-receptors. This possibility is difficult to rule out with present data, but such a mechanism does not appear to operate in dog carotid or guinea-pig vas deferens (O'Donnell et al., 1981) or in guinea-pig aorta in which antagonism of H_1-receptors by photolyzed AAH is easily achieved.

Taken together, the present results suggest that a difference exists in the H_1-receptors of dog trachealis and guinea-pig aorta insofar as their interaction with *photolyzed* AAH is concerned. The H_1-receptors of the two tissues are not as different in other respects, i.e., the affinities of the receptors for diphenhydramine and nonphotolyzed AAH, and the diphenhydramine/nonphotolyzed AAH potency ratios are quite similar (table 3).

A tentative explanation of our findings may be made if it is assumed that the topologies of the H_1-receptors in the two tissue are different. Many currently available H_1- and H_2-receptor agonists and selected H_2-antagonists contain small (i.e., methyl) structural additions to the imidazole portion of the molecule. It follows that the imidazole moiety of AAH is responsible for recognition by the H_1- and H_2-receptors and the resultant affinity for the compound. The bulky azidonitrilanilino grouping on the imidazole portion confers low affinity ($K_B \simeq 10^{-5}$ M) of AAH for H_1-receptor. In the guinea-pig aorta, the region of the receptor which recognizes and binds the imidazole portion of the molecule may be adjacent to or surrounded by chemical groupings into which the highly reactive aryl nitrene intermediate produced by photolysis can covalently insert. The domains around the H_1-receptor recognition site might be similar in guinea-pig vas deferens and dog carotid. According to this hypothesis, the chemical groupings into which the aryl nitrene inserts would be lacking in the dog trachealis. This hypothesis takes into account several aspects of the results: (1) The H_1-receptors of guinea-pig aorta and dog trachealis are quite similar in terms of their relative affinities for diphenhydramine and nonphotolyzed AAH; that is, the presence or absence of a moiety available for insertion by the aryl nitrene has no influence on equilibrium competitive binding of the antagonists to the recognition site. (2) The site which recognizes AAH, to which nonphotolyzed AAH binds before photolysis, is different from the moiety which is available for insertion by the aryl nitrene. If, as in the dog trachealis, the insertion site is not available for the formation of a covalent attachment during photolysis, the AAH freely dissociates from the receptor after washing the agent from the bath; no residual, irreversible antagonistic effects are subsequently observed.

A comparison between the antagonisms produced by 3×10^{-5} M and 10^{-5} M photolyzed AAH reveals an interesting and unpredictable paradox. 3×10^{-5} M AAH produced, after photolysis, an 11.5-fold shift of the histamine concentration-response curve of guinea-pig aorta to the right of control, and a 38% reduction in the maximum response (fig. 1 and table 1A). These results are in keeping with those expected of a nonequilibrium competitive antagonism involving inhibition of spare receptors (see for comparison, Kwok and Moore, 1980). However, 10^{-5} M AAH, after photolysis, produced a 19% reduction of maximum response to histamine but the EC_{50} for histamine was not significantly increased (table 1B). If spare histamine receptors exist, then an increase in EC_{50} should have preceded a reduction in maximum response as AAH concentration was increased. The reason for this nonclassical nonequilibrium competitive antagonism is not understood.

Photolyzed AAH produced no other pharmacological effect in guinea-pig aorta except histamine antagonism. Nonphotolyzed AAH, on the other hand, antagonized K^+-induced responses as well. In dog trachealis, nonphotolyzed AAH exerted only an antihistamine effect. Furthermore, nonphotolyzed AAH potentiated contractile responses of guinea-pig vas deferens to ATP and norepinephrine (O'Donnell et al., 1981). Thus, nonphotolyzed AAH produces diverse and seemingly unrelated pharmacological effects in different smooth muscle preparations. It is of interest to note that the unknown mechanism responsible for these effects of nonphotolyzed AAH is not irreversibly activated when AAH is photolyzed. That is, pho-

tolysis of AAH produces only one effect in sensitive tissues, namely, antagonism of responses to histamine mediated via H_1-receptors.

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