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(EC_{50} with epithelium: 1.11 ± 0.48 μM control, 2.01 ± 0.71 μM with saxitoxin; EC_{50} without epithelium: 2.83 ± 0.55 μM control, 2.05 ± 0.58 μM with saxitoxin). Also, 1 μM saxitoxin did not alter contractions of parenchymal strips to cumulatively added acetylcholine (EC_{50}: 3.47 ± 1.06 μM, control; 3.98 ± 1.19 μM with saxitoxin), histamine (EC_{50}: 0.83 ± 0.24 μM, control; 0.60 ± 0.13 μM with saxitoxin); or of aorta strips to norepinephrine with endothelium (EC_{50}: 1.78 ± 0.80 μM, control; 0.74 ± 0.21 μM with saxitoxin) or without endothelium (EC_{50}: 2.18 ± 0.78 μM, control; 1.42 ± 0.62 μM with saxitoxin). Thus, saxitoxin did not significantly alter contractile activity of airways or large arteries *in vitro*. Keywords: cont'd — dinoflagellate algae; physiological effects; toxins; (x-)

LACK OF AN EFFECT OF SAXITOXIN ON THE CONTRACTILITY OF ISOLATED GUINEA PIG TRACHEA, LUNG PARENCHYMA AND AORTA

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INTRODUCTION

Saxitoxin, a low molecular weight (294), nonprotein neurotoxin produced by dinoflagellate algae, is responsible for paralytic shellfish poisoning. It closely resembles tetrodotoxin in its interference with sodium conductance in excitable membranes but differs somewhat in other actions (KAO, 1972).

Among its other effects are those on the vasculature. From experiments in the innervated, cross-perfused hindleg of the cat, NAGASAWA *et al.* (1971) concluded that saxitoxin causes a direct vascular relaxation in doses up to 1.5 µg/kg and, at higher doses, a blockade of vasoconstrictor nerves. Tetrodotoxin has a more potent hypotensive effect *in vivo* (KAO, 1966, 1972), but no direct vasorelaxant action (FEINSTEIN and PAIMRE, 1968). Therefore, we determined that studies of the effects of saxitoxin on isolated vessels would be of interest.

Saxitoxin also affects the respiratory system. It caused a transient (1-3 min) increase in airways resistance of approximately 30% after slow infusion (0.315 µg/kg/min) of approximately 3 µg/kg into awake guinea pigs (FRANZ and LECLAIRE, 1988) and may thus cause an airways constriction of brief duration. Although death resulted from respiratory failure in these animals, it was probably not due to direct effects on the airways. Effects of saxitoxin on isolated airways have not been described.

As an animal model for both airway and vascular effects of saxitoxin, guinea pigs were chosen. The guinea pig trachea is widely used as a suitable model for human large and central airways (MUCCITELLI *et al.*, 1987). The guinea pig parenchymal strip can be used to evaluate the responses of the peripheral airways (DRAZEN and SCHNEIDER, 1978). In order to examine saxitoxin's effects on blood vessels of the same species, contractility of guinea pig thoracic aorta was also investigated. Because tracheal epithelium and vascular endothelium can release factors that modulate the tension of smooth muscle, experiments with and without these cell layers were carried out.

MATERIALS AND METHODS

Male, barrier-raised, Hartley albino guinea pigs weighing 253-405 g (Charles River Labs, Wilmington, MA) were housed in a bio-safety enclosure (Airoclean Engineering, Inc., Edgemont, PA). After stunning (SMITH, 1986) and exsanguination, lungs, trachea and the upper one-half of the aorta were removed quickly and placed into aerated Tyrode's solution.

Saxitoxin (Source: Dr. Sherwood Hall, FDA, Washington, D.C.), 94% pure as measured by HPLC, was prepared as a 10 or 100 µM solution in 0.001 N acetic acid and stored at -25° until the day it was used, then thawed immediately before dilution and use. Other drugs employed (acetylcholine chloride, carbachol, histamine

dihydrochloride, and norepinephrine bitartrate) were prepared as stock solutions, frozen, then diluted fresh each day.

All tissues were carefully cleaned of fat and connective tissue. The trachea, from the larynx to the carina, was cut into rings 4-5 mm wide. The upper half of the thoracic aorta was cut into rings 3-4 mm wide. Two loops of silk thread were tied through each tissue ring. The lower loop was attached to a fixed hook and the upper loop was connected to an isometric force transducer for tension measurements. In half of the experiments, the epithelium or endothelium was removed from the tissue rings by rubbing with a moist cotton swab. The rings which were not rubbed had intact epithelium or endothelium, respectively, while the rubbed rings had approximately 90% of the epithelium and all endothelium removed, as confirmed by sectioning, staining and microscopic examination. Strips of pulmonary parenchyma approximately 1.5 x 1.5 x 12 mm were cut from the margin of lung lobes. These were tied at both ends and attached as described above.

Tissues were suspended in aerated Tyrode's solution (38°) which was continuously bubbled with 95% O_2 - 5% CO_2. Initially, 1 g of tension was placed on the trachea and aorta rings while 300 mg was placed on the parenchymal strips. After the tissues were stretched for a few minutos, they were contracted by exposure to the maximal concentration of agonist to which they would be later exposed. Once a maximal response was obtained, the tissues were washed and allowed to equilibrate for 1 hr. Cumulative

concentration-response curves were then obtained by successive addition of agonist to the bath according to the method of VAN ROSSUM (1963). Each addition of agonist was made after the response to the previous concentration had reached a plateau. After 45 min of periodic washing, cumulative concentrations of saxitoxin up to 0.1 μM or equal volumes of vehicle were added to each bath. Fifteen minutes later, the original agonist was again cumulatively added in the presence of saxitoxin. Agonists employed were 0.01-10 μM carbachol (trachea), 0.1 to 100 μM acetylcholine or 0.1 to 10 μM histamine (parenchyma), and 0.03-10 μM norepinephrine (aorta). Effects of saxitoxin on resting tension of the tissues, and on the tension of tissues contracted with the highest concentration of the agonists were also determined. Responses of saxitoxin-exposed tissue strips were corrected for changes in tissue sensitivity noted in control strips. To evaluate the effects of saxitoxin on tissue responses to agonists, the concentration of agonist required to produce a contraction equivalent to 50% of the maximal contraction produced by that agonist (EC_{50}) was calculated graphically for responses in the presence and absence of saxitoxin. Comparisons of these values were made by Student's t test with $P < 0.05$ considered significant.

RESULTS

Guinea pig tracheal rings contracted in a similar dose-response manner after cumulative addition of carbacnol (0.01-10μM; Fig. 1), both in the presence and absence of epithelium. The

EC_{50} for carbachol on tracheal rings, with and without epithelium, was not significantly altered by saxitoxin (Table 1). Finally, the rate of relaxation of tracheal rings contracted by carbachol was not altered by saxitoxin (data not shown).

Similarly, cumulative addition of agonist (acetylcholine or histamine) to baths containing lung parenchymal strips resulted in the expected dose response relationship (Fig. 2). The maximal contraction due to histamine, however, was slightly higher than the maximal response obtained with acetylcholine, which is in agreement with DRAZEN and SCHNEIDER (1978). More importantly, however, saxitoxin did not alter the dose-response relationship to either agonist, as evidenced by the dose-response curves and EC_{50}s (Fig 2, Table 1).

Norepinephrine was used as the agonist in the aortic ring preparation and produced the expected dose-response curve, both with endothelium present and removed. Again, saxitoxin failed to alter the contractile response to the agonist (Fig. 3, Table 1).

Finally, cumulatively added concentrations of 1 pM to 0.1 μM saxitoxin did not alter the resting tension of tracheal or aorta rings or lung parenchymal strips, nor did 0.1 μM saxitoxin reduce the tension of tracheal rings maximally contracted by 10 μM carbachol, parenchymal strips contracted by 100 μM acetylcholine or by 10 μM histamine; or of aorta rings contracted by 10 μM norepinephrine (data not shown).

DISCUSSION

STIMLER-GERARD (1986) reported that tetrodotoxin does not alter contractions of guinea pig lung parenchyma to either acetylcholine or histamine. We report no effect of saxitoxin on cumulative, agonist-induced contraction of airway smooth muscle. Because carbachol-induced contractions of parenchymal strips are blocked by atropine and histamine-induced contractions are blocked by mepyramine (DRAZEN and SCHNEIDER, 1978), our data indicate a lack of selective anticholinergic, antihistaminic (at the H_1 receptor) or non-selective relaxant effects by saxitoxin in these airway tissues. This was further verified by a lack of effect either on the resting tension or on the tension of strips maximally contracted with either histamine or acetylcholine (not shown).

In the concentrations used here, saxitoxin did not affect tracheal rings *in vitro*. The tracheal ring and lung parenchymal strip are considered good models of central airways (primarily responsible for airways resistance) and peripheral airways (which influence pulmonary compliance), respectively (DRAZEN and SCHNEIDER, 1973). Thus, the transient increase in airway resistance in awake guinea pigs previously noted (FRANZ and LECLAIRE, 1988) was likely due to neural effects rather than direct effects on airways smooth muscle.

Results of studies with aorta rings demonstrated that saxitoxin did not have a direct relaxant effect on the guinea pig

aorta. Furthermore, the lack of an effect on norepinephrine-stimulated contraction indicates no involvement of alpha-adrenergic mechanisms. It is well known, however, that small resistance vessels can respond to agents differently than large arteries. Therefore, these data should not be taken as contradicting previous findings of a direct vasodilation by saxitoxin (NAGASAWA *et al*, 1971), nor as supporting the work of those who found no evidence for a direct vasodilating effect (FEINSTEIN and PAIMRE, 1968), as those data were obtained in isolated vascular beds which react differently than does a large artery.

In conclusion, it is generally believed that axonal blockade and, possibly relaxation of end-organ muscle are the predominant causes of saxitoxin-induced hypotension and respiratory failure *in vivo* (KAO, 1972). These data demonstrate that saxitoxin did not directly affect the smooth muscle of airways or large blood vessels *in vitro*.

Acknowledgements—The opinions and assertions contained herein are the private views of the authors and are not to be construed as official of as reflecting the views of the Department of Defense. In conducting the research described in this report, the authors adhered to the Guide for Laboratory Animal Facilities and Care as promulgated by the Committee on the Guide for Laboratory Animal Resources, NAS/NRC.

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TABLE 1.AGONIST EC_{50}S (μM) IN GUINEA PIG TRACHEAL AND AORTA RINGS AND LUNG PARENCHYMAL STRIPS BEFORE AND AFTER ADDITION OF 0.1μM SAXITOXIN TO THE BATHING MEDIA*

	CONTROL	SAXITOXIN
TRACHEAL RING (Carbachol)		
Epithelium present	1.11 ± 0.48	2.01 ± 0.71
Epithelium removed	2.83 ± 0.55	2.05 ± 0.58
LUNG PARENCHYMA		
Acetylcholine	3.47 ± 1.06	3.98 ± 1.19
Histamine	0.83 ± 0.24	0.60 ± 0.13
AORTA RING (Norepinephrine)		
Endothelium present	1.78 ± 0.80	0.74 ± 0.21
Endothelium removed	2.18 ± 0.78	1.42 ± 0.62

*Values are the mean ± SEM for 4-10 tissue strips.

No significance at $P<0.05$.

LEGENDS FOR FIGURES

FIG. 1. CONTRACTION OF GUINEA PIG TRACHEAL RINGS TO CARBACHOL. Each point represents the mean ± SEM of 6-9 observations. 0 = before and ● = after saxitoxin.

FIG. 2. CONTRACTIONS OF GUINEA PIG PARENCHYMAL STRIPS TO ACETYLCHOLINE OR TO HISTAMINE. Each point represents the mean ± SEM of 4-10 observations. 0 = before and ● = after saxitoxin.

FIG. 3. CONTRACTIONS OF GUINEA PIG AORTA RINGS TO NOREPINEPHRINE. Each point represents the mean ± SEM of 5-7 observations. 0 = before and ● = after saxitoxin.

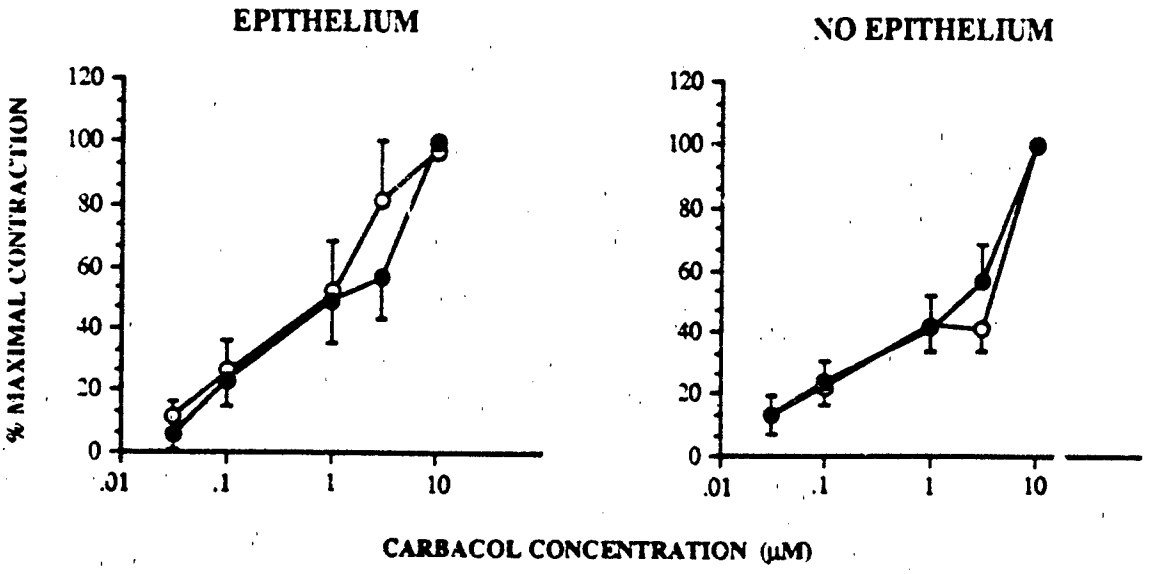
EPITHELIUM
NO EPITHELIUM
% MAXIMAL CONTRACTION
120
100
80
60
40
20
0
.01
.1
1
10
CARBACOL CONCENTRATION (μM)

Fig 1

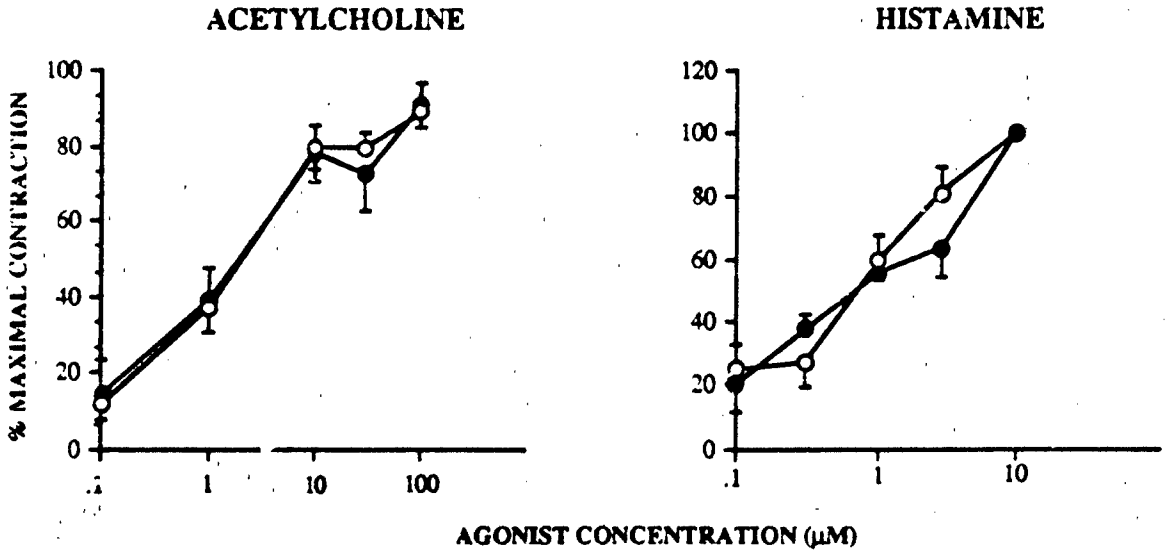
ACETYLCHOLINE
HISTAMINE
% MAXIMAL CONTRACTION
100
80
60
40
20
0
1
10
100
120
.1
AGONIST CONCENTRATION (μM)

Fig 2

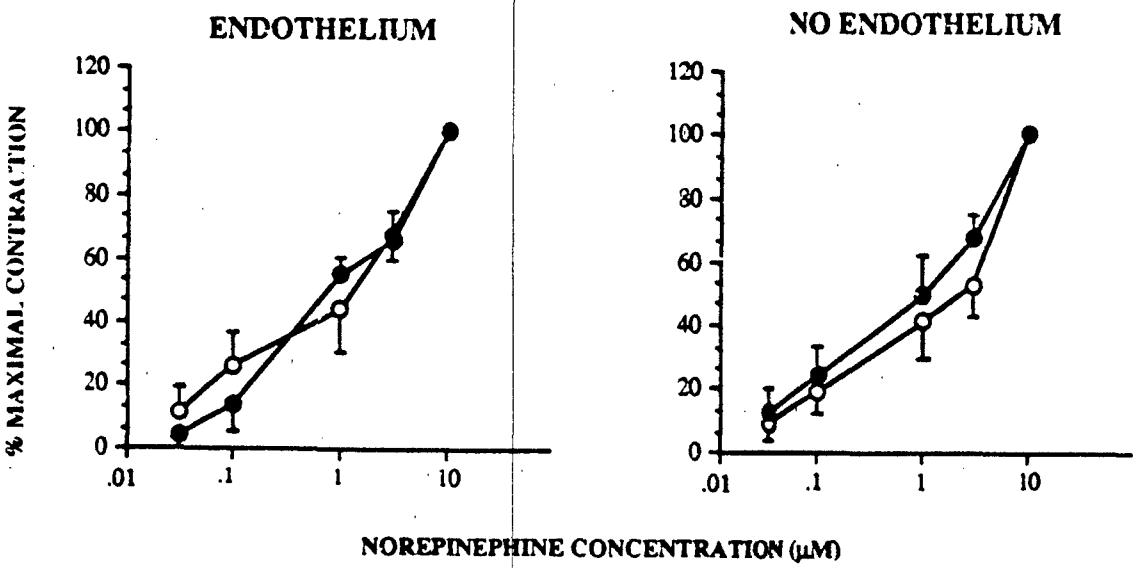
ENDOTHELIUM
NO ENDOTHELIUM
% MAXIMAL CONTRACTION
120
100
80
60
40
20
0
.01
.1
1
10
NOREPINEPHINE CONCENTRATION (μM)

Fig 3