IN VITRO STUDIES ON CONTROL OF FLUID SECRETION BY ISOLATED SALIVARY GLANDS OF THE LONE STAR TICK AMBLYOMMA AMERICANUM (LINNAEUS)

By

GLEN RAY NEEDHAM

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Southwestern Oklahoma State University

Weatherford, Oklahoma

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Thesis Approved:

John R. Sauer
Thesis Advisor

Calvin G. Beames, Jr.

J. Alexander Hair

N. D. Durham
Dean of the Graduate College

PREFACE

This investigation was designed to use an *in vitro* technique to elucidate possible mechanisms of control of fluid secretion by salivary glands of the feeding female lone star tick, *Amblyomma americanum* (L.). Various drugs and/or inhibitors were incorporated into the bathing medium surrounding isolated glands with the effects on fluid secretion being determined by changes in the rate of fluid secreted. Potential stimulants used were cyclic AMP, theophylline, pilocarpine, glutamate, 5-hydroxytryptamine and noradrenaline; adrenaline served as a control for all experiments to determine secretory competence of glands. Ouabain, harmaline and 2,4-dinitrophenol were potential inhibitors incorporated into the bathing medium containing adrenaline, with results compared to a control in which adrenaline was present without the inhibitor.

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This manuscript is dedicated to my family whose understanding and patience encouraged me to take this first step foreward into a career in science.

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CHAPTER I

INTRODUCTION

Ticks concentrate ingested blood by eliminating excess salt and water. Ixodid (hard) ticks utilize their paired salivary glands to excrete excess ions and water while still attached and feeding on the host (Gregson, 1967; Kaufman and Phillips, 1973a, b, c; Sauer and Hair, 1972; Tatchell, 1967; 1969).

Kaufman and Phillips (1973c) suggested that in Dermacentor andersoni (Stiles), salivation occurs by means of a secretory rather than a filtration-resorption mechanism and that control of fluid secretion may be neural rather than hormonal, citing the effectiveness of catecholamines in stimulating fluid secretion. They also suggested the possible role of a chloride pump for driving secretion. Hsu and Sauer (1975) have shown that chloride is higher in concentration in the oral secretion (saliva) than in the hemolymph of engorging female Amblyomma americanum (L.). Therefore, studies on control of ^{36}Cl uptake by salivary glands of A. americanum were initiated (Sauer et al., 1974). Results supported the findings of Kaufman and Phillips (1973b) concerning the importance of catecholamines, but cyclic AMP was found to increase ^{36}Cl uptake in A. americanum, in contrast to the inability of this same drug to stimulate fluid secretion by glands of D. andersoni (Kaufman and Phillips, 1973b).

Differences in the effectiveness of cyclic AMP to stimulate

glandular function in these two species prompted us to additionally investigate the effects of drugs and inhibitors on tick salivary fluid secretion in the lone star tick.

CHAPTER II

REVIEW OF THE LITERATURE

The lone star tick, Amblyomma americanum (L.), is an obligate temporary parasite of most wild and domestic animals, birds and man (Cooley and Kohls, 1944). Its geographical distribution includes an area as far west as west-central Texas, north to Missouri, then east in a broad belt to the Atlantic coast (Hair and Howell, 1970).

The lone star tick is important both economically and medically. Its impact is shown by the slow development of infested areas; in addition, the damage done to domestic animals (i.e., cattle, horses, sheep) can only be estimated. Medically, ticks cause a great deal of annoyance due to their bite alone. This ticks grim reputation stems a great deal from its ability to transmit diseases such as Rocky Mountain Spotted Fever (RMSF), tularemia, Q-fever and tick paralysis (Hair and Howell, 1970). Transmission of RMSF from the engorged female to her eggs (transovarian) and from stage to stage (transtaadial) has been demonstrated (James and Harwood, 1969).

This tick is a three-host species with each instar (larvae, nymph, adult) requiring a separate bloodmeal on a different host. Ixodid or "hard" ticks have the capability to imbibe large quantities of blood during feeding, with excess water and electrolytes eliminated back into the host via the salivary glands (Gregson, 1967, Kaufman and Phillips, 1973a, b, c; Sauer and Hair, 1972; Tatchell, 1967; 1969). The

subsequent bloodmeal is concentrated in the gut, where it is digested and passed into the hemolymph. The engorged female incorporates these nutrients into oocytes for their development and yolk production (vitellogenesis). Immature stages utilize these digested products for tissue building preparation for subsequent molting to the next instar.

Bloodsucking arthropods, which ingest large volumes of blood during feeding, must regulate concentrations of solutes and water within their various internal body compartments. In mosquitoes, tsetse flies, and many hemipteran insects the excess water is eliminated via the Malpighian tubules. In ixodid ticks Malpighian tubules are not active until after detachment from the host (Balashov, 1967; Till, 1961) and they perform primarily an excess nitrogen excretory function. Gregson (1960) reported that in D. andersoni a dilute clear oral secretion was responsible for returning excess water from the ingested bloodmeal back into the host.

To test this hypothesis, Tatchell (1967) injected tritiated water into the hemocoel of feeding adult cattle ticks, Boophilus microplus (Canestrini), subsequently recovering 26 percent of the radioactivity from the host and its excreta. He concluded that during engorgement large amounts of water which have passed across the gut epithelium into the hemolymph are returned to the host. Tatchell (1969) later indicated that the salivary secretory mechanism is able to exercise some regulatory control over hemolymph osmotic pressure and the ionic concentration in the engorged cattle tick. Of the total meal imbibed by female D. andersoni during the normal adult feeding cycle, about 80 percent is excreted. Of the total water excreted by the tick, 75 percent is removed by salivation, three percent is evaporated from the

integument and spiracles, and the remainder is lost via the anus (Kaufman and Phillips, 1973a).

The salivary glands of ixodid ticks play several important roles in addition to maintenance of salt and water balance. It is thought that the salivary glands produce a secretion which solidifies into a cement sheath to hold the mouthparts firmly in the hosts skin while the tick is feeding (Moorhouse and Tatchell, 1966). The cement is apparently nonantigenic and thus there is no inflammatory response in the area of direct contact with skin cells. By surrounding the mouthparts the sheath also prevents irritation which might be caused by shifting or movement of the mouthparts (Balashov, 1967)

Many ticks are also capable of producing a salivary discharge into the wound which prevents coagulation in the preoral cavity (Gregson, 1960) and intensifies hemorrhage in damaged capillaries. Saliva may also cause lysis of surrounding cells and tissues, as the mouthparts are not adapted to allow passage of solid food or very large fragments (Balashov, 1967). A paralytic toxin may also be synthesized by the glands (Gregson, 1957; Ross, 1926).

For salivary glands to function in so many capacities there must be several cell types present in the glandular alveoli. One might also suspect the presence of one or more control mechanisms, whereby the tick could turn on and off the various processes carried our by the glands. Although salivary glands in mammalian systems perform no osmoregulatory function, some correlation may possibly be drawn. In a review article by Schneyer et al. (1972) the following points were made concerning salivary composition and its regulation (in mammals). Secretion of saliva from the salivary glands is generally elicited in response to

stimulation of autonomic innervation to the glands or in response to drugs that mimic the actions of autonomic innervation. Although stimulation of the sympathetic innervation can result in secretion of saliva, salivary flow results mainly from stimulation via parasympathetic nerves. The amount and composition of the saliva are related to the kind of autonomic secretomotor activity involved in evoking secretion. Saliva evoked by action of adrenergic mediators is generally higher in organic content than saliva evoked by cholinergic stimulation (Langstroth et al., 1938) and higher also in its content of certain inorganic salts (Schneyer and Schneyer, 1960).

Kaufman and Phillips (1973b) demonstrated in D. andersoni that salivation occurs by means of a secretory rather than filtration-resorption mechanism and that fluid secretion is probably neural rather than hormonal, the transmitter substance being catecholaminergic in nature (i.e., adrenaline, noradrenaline and dopamine). They found that adrenaline could be used to stimulate the salivary glands to secrete in vitro and in vivo.

The method used by Kaufman and Phillips (1973b) to induce salivation in vitro was called a "pulse" method. Glands were left in an adrenaline-containing Ringer solution only until a maximum rate of secretion was acheived. At that point the glands were washed several times with Ringer's solution free of adrenaline, at which time the secretory rate fell to zero. A "pulse" of adrenaline with rest periods between caused an increase in duration of secretion as compared to glands which were continually bathed in adrenaline. The rate of decay with this particular method became a linear function of time. Experimentors have incorporated a similar in vitro technique to study insect

salivary glands and Malpighian tubules (Berridge and Prince, 1972; Maddrell, 1972; Maddrell and Klunsuwan, 1973; Ramsey, 1954).

Researchers (Kaufman and Phillips, 1973c) have suggested that chloride may be the driving force responsible for fluid secretion in D. andersoni. Data on the lone star tick indicates a high saliva to hemolymph ratio of both Na and Cl ions (Hsu and Sauer, 1975), which suggests transport against the concentration gradient. If uptake is indeed responsible for fluid movement, then one might speculate that control of fluid secretion is dependent on controlling uptake of chloride. Fluid transport has been described by the standing gradient hypothesis (Diamond and Bossert, 1967; 1968). This theory suggests a flow iso-osmotically across a fluid transporting epithelium in which solutes are pumped from the cell's basal infoldings, creating an area of low solute concentration in the channel and high concentration in the cell. Before the solute diffuses away, water enters the cell down an osmotic gradient and more body fluid water moves down the channel to take its place. When a steady state is reached, ion pumps initially create a high solute concentration in the apical infoldings and water leaves the cell in response to this gradient, pushing solute and fluid toward the lumen. This fluid appears to flow between two equi-osmolar solutions, but, in fact is flowing along local osmotic gradients within the cell.

Salivary glands in D. andersoni have several different types of acini, but one in particular, type III (Meredith and Kaufman, 1973), has cells with extensively infolded basal and lateral plasma membranes. These morphological characteristics conform well to the geometrical requirements of the standing gradient hypothesis. Mitochondria are

also closely associated with the infoldings, an arrangement which is generally believed to bring energy stores of the cell near to the ion pumps which are responsible for active transport (Meredith and Kaufman, 1973). Similar morphological features have been found in the glands of lone star ticks (Barker[1]).

Chloride uptake studies by Sauer et al. (1974) on A. americanum support to some extent the theory of Kaufman and Phillips (1973b) that control of fluid secretion may be neural rather than hormonal and that the transmitter substance is a catecholaminergic substance, the evidence being that adrenaline, noradrenaline and dopamine had stimulatory effects on chloride uptake and high concentrations of pilocarpine and glutamate had no significant effect. Sauer et al. (1974) found that the hormonal "second messenger" cyclic AMP stimulated a significant uptake of ^{36}Cl, but, Kaufman and Phillips (1973b) could show no stimulation of fluid secretion when glands of D. andersoni were incubated with cyclic AMP.

Other experimentors (Coons and Roshdy, 1973; Coons[2]) have indicated the presence of axons with neurosecretory material in close association with the acini in various ticks including A. americanum. It was suggested (Sauer et al., 1974) that the catecholaminergic molecules could stimulate these axons to release their neurosecretory material locally, thus affecting the salivary glands.

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[1]Barker, R. W., Personal Communication, Georgia Experiment Station, Tifton, Georgia, 1973.

[2]Coons, L. B., Personal Communication, Department of Entomology, Mississippi State University, Mississippi State, Mississippi, 1974.

CHAPTER III

MATERIALS AND METHODS

Experimental Animals

Ticks used in all experiments were female lone star ticks close to repletion on the host and in a state of rapid feeding. Larval and nymphal stages were reared on rabbits as described by Patrick and Hair (1975). Equal numbers of both male and female unfed adults (30-40) were placed in an area on the dorsolateral side of sheep, this area had been previously shorn (six inch circle) and washed. Orthopedic stocking of equal size to the shorn area was cemented in place utilizing Borden^{R} contact cement, the open end of the cell being secured with a rubber band. Ticks were allowed to feed to near repletion (rapidly feeding), removed and taken to the laboratory for experimentation within one hour following removal from the host.

In Vitro Technique

Salivary glands were removed from female A. americanum ticks undergoing the rapid last phase of engorgement on the host sheep. Dissection and bathing media preparations contained the following constituents as suggested by Rahacek and Brzostowski (1969): NaCl, 10.00g; $NaH_2PO_4 \cdot H_2O$, 2.35g; $Na_2HPO_4 \cdot 7H_2O$, 2.35g; $KHCO_3$, 1.92g; $CaCl_2$, 0.39g; $MgSO_4$, 0.34g; glucose, 5.88g; inositol, 0.47g and bovine albumin, 0.10g; diluted to one liter volumetrically with deionized water. Salines had a pH of 6.8

and freezing point depression of -0.777°C. All solutions were pre-oxygenated with one hundred percent oxygen for thirty minutes prior to use in experiments. Osmolarity determinations of the salines used in the study were measured with a Clifton Technical Nanoliter Osmometer, expressed as the freezing point depression (Δ°C), being sensitive to the nearest $\pm$ 0.001°C (Frick and Sauer, 1973). The pH of the salines was determined by the Coleman Model 39 pH Meter.

To isolate the salivary glands the dorsal cuticle was carefully removed by making a circular incision around the tick laterally using a sharp, single edge razor blade. With forceps and probe the gut, reproductive, nervous and tracheal systems along with any adhering tissue were carefully removed to fully expose the glands and ducts. A fine pointed probe was used to detach the main salivary duct from the sclerotized mouthparts close to the salivarium. A drop of saline was added to a disposable petri dish covered with black wax and filled with liquid paraffin. The gland was rinsed and placed in the drop of saline, which was secured by a conical depression in the wax. The main duct was located and manipulated by a fine insect pin and drawn from the drop of Ringer saline to the surrounding liquid paraffin. During glandular secretion the fluid accumulated on the insect pin, where it was collected with a finely tapered capillary. After collection and transfer to a distant part of the liquid paraffin, the spherically shaped droplet's diameter was measured with a dissecting microscope equipped with an ocular micrometer and its volume determined by making the appropriate mathmatical calculation.

Experimental Protocol

In preliminary experiments, adrenaline-saline solutions stimulated salivary fluid secretion at adrenaline concentrations ranging from 10^{-3}M to 10^{-6}M. Consistent success in stimulating salivation was achieved by incorporating 10^{-5}M adrenaline in the gland's bathing medium.

All experiments were divided into three phases, pre-experimental, experimental and post-experimental. The pre-experimental phase included incubation of the glands with 10^{-5}M adrenaline, which served the purpose of determining secretory competence of the glands. Salivary glands were exposed to fresh or new drug-Ringer solution and rinsed at precisely the same times in all phases of the experiment (eight minute intervals). This made it possible to make statistical comparisons between controls and experimental treatments. Glands were incubated in the drug-Ringer saline for five of the eight minutes, the remaining three minutes were used for measuring the secreted droplet and rinsing the gland. The experimental phase tested the effects of various potential glandular stimulants and/or inhibitors of secretion. Two controls were used for each category. The stimulating drugs were added with the Ringer's saline in the same manner as adrenaline-saline solutions in the pre-and post-phases; however, potential fluid inhibitors were added with the presence of adrenaline because the glands will not secrete in absence of a stimulating factor.

Method of Analysing Data

As previously stated, controls for both drug categories (stimulant or inhibitor) were established. The method for analysing the data as compared to controls was as follows: after noticeable variations of

maximum rates of fluid secretion, it was decided to compare changes in glandular response rather than actual responses. This method was used because the trend or type of response to a particular drug was usually consistent. Variations in intensity may have been due to differences in physiological states of individual glands even though all were removed from rapidly engorging females. The analysis was made by averaging the four treatments with the adrenaline in the pre-experimental phase and the three treatments with the test drug in the experimental phase. The two averages were subtracted to give the change in response. The average change exhibited by the experimental drug was compared to the average change shown by its appropriate control. The t test was used to detect significance in rate changes at the $P < 0.05$ level.

Source of Chemicals or Drugs

The drugs harmaline, 5-hydroxytryptamine (serotonin), theophylline, L-glutamate and adenosine-3',5'-monophosphate (cyclic AMP) were purchased from Nutritional Biochemicals Corp. K&K Laboratories was the source of L-adrenaline, L-noradrenaline, ouabain and pilocarpine nitrate. N^6-2'-O-dibutyryl adenosine-3',5'-monophosphate (dibutyryl cyclic AMP) was obtained from the Sigma Co. and 2,4-dinitrophenol (DNP) from Fisher Scientific Co.

CHAPTER IV

RESULTS

Effects of Drugs and Pharmacological Agents

Figure 1 depicts salivary secretion when glands were incubated in the pre- and post-experimental phases of the experiment with a Ringer's saline containing 10^{-5}M adrenaline. The rate reached a maximum average of 97 nl/min in the pre-experimental phase. In the experimental phase, which served as the control for the following experiments, secretion rapidly dropped to zero when adrenaline was deleted from the bathing medium. Results show that glandular function can be restored when adrenaline is reapplied in the post-experimental phases of the experiments, rates averaged 71 $\pm$ 28, 0 and 49 $\pm$ 32 ($\pm$ means S.E.M.), respectively (Table I).

In contrast to the above, cyclic AMP had a stimulatory effect when added during the experimental portion of the determination, causing glands to secrete at an average rate of 124 $\pm$ 42 nl/min (Table I). This contrasts with results obtained by Kaufman and Phillips (1973b), who were unsuccessful in getting glands of D. andersoni to secrete after administering cyclic AMP to the bathing medium. However, these authors did not pre-incubate the glands with adrenaline, as was done in our experiments. Because of this difference, I tested the ability of cyclic AMP to initiate glandular secretion in the absence of prior adrenaline stimulation (Figure 2). I, too, was unsuccessful in obtaining secretion

under these conditions. To verify that the glands were competent, adrenaline was added to the bathing medium following cyclic AMP administration and without exception, the glands secreted after addition of the drug (Figure 2).

To further test the possible role of cyclic AMP in glandular secretion, theophylline, an inhibitor of the enzyme phosphodiesterase, was added during the experimental phase of experiments. It, too, stimulated glandular secretion (Table I). However, like cyclic AMP, no appreciable rate of secretion was achieved when theophylline was added without prior adrenaline stimulation (Figure 2). Additions of dibutyryl cyclic AMP was lengthened because evidence points to the requirements for intracellular esterases to remove the butyryl substituent from the 2'-position before this derivative can become active (Berridge and Prince, 1972).

When glands were incubated with the parasympathetic drug pilocarpine, no secretion during the experimental phase was noted (Table I).

Noradrenaline differs from adrenaline by only one methyl group, yet it appears to be an even more potent stimulant of salivary secretion (Table I). Murdock (1971) has stated that the evidence for the presence of adrenaline in arthropods is minimal. Glutamate, even at high concentrations, showed no potential to cause glandular secretion (Table I). Salivary glands of the adult blowfly, Calliphora erythrocephala (Meigen), are receptive to both cyclic AMP and 5-HT (Berridge and Patel, 1968; Berridge and Prince, 1972); however, glands from the lone star tick did not secrete when 5-HT was present in the bathing medium (Table I).

Figure 1. Control for Potential Stimulants of Salivary Fluid Secretion With Glands of the Lone Star Tick Exposed to Adrenaline-Saline Solutions in Pre- and Post-Experimental Phases. Secretory Rates During the Experimental Phase Served as the Control for Testing Potential Stimulatory Drugs. (↑) Removal; (↓) Addition of Fresh Bathing Medium. Each Point Represents the Average of Four Separate Experiments.

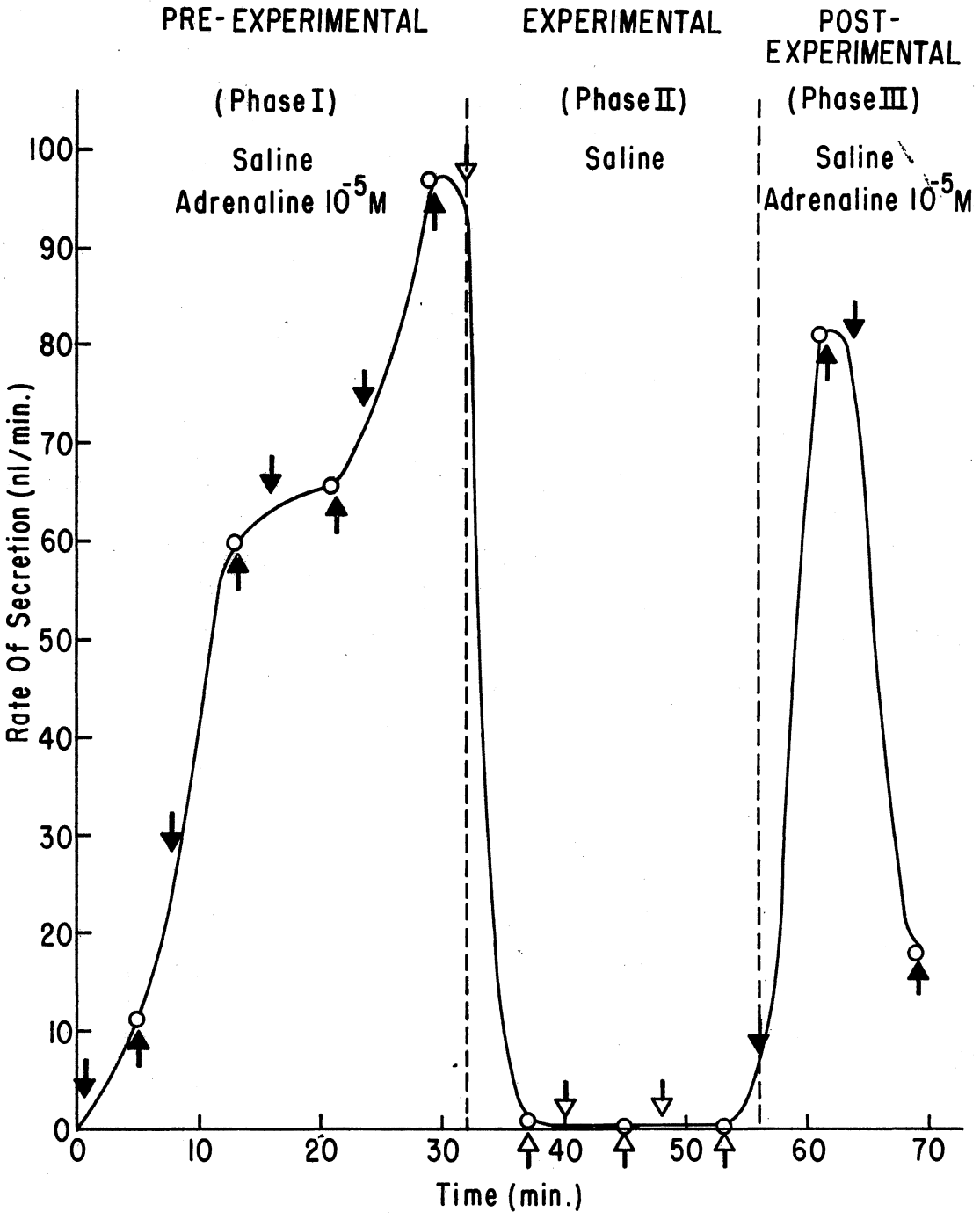

PRE-EXPERIMENTAL
(Phase I)
Saline
Adrenaline 10^{-5}M
EXPERIMENTAL
(Phase II)
Saline
POST-EXPERIMENTAL
(Phase III)
Saline
Adrenaline 10^{-5}M
Rate Of Secretion (nl/min.)
Time (min.)
0
10
20
30
40
50
60
70
80
90
100

TABLE I

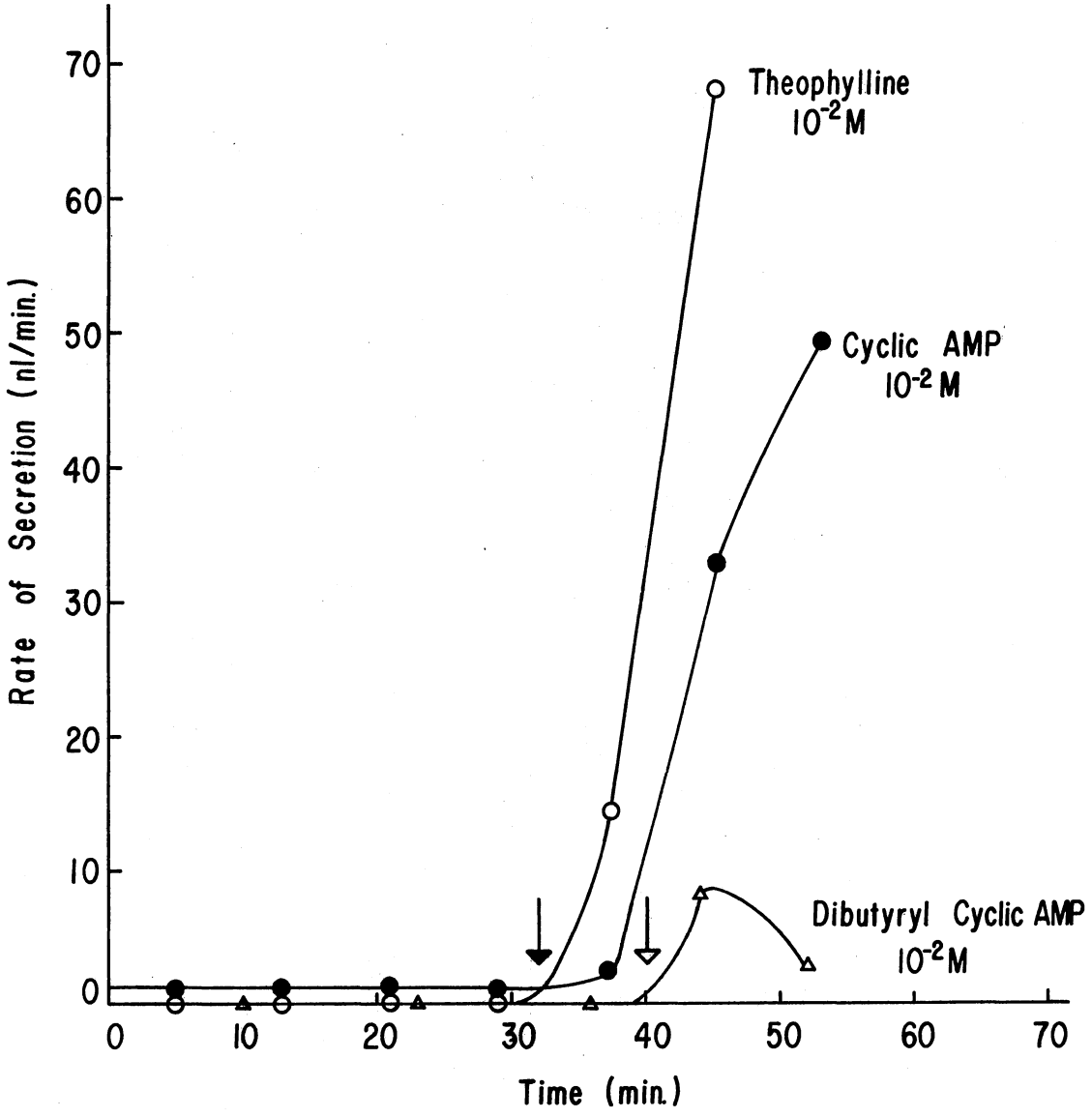
EFFECTS OF POTENTIAL STIMULANTS ON RATES OF FLUID SECRETION IN VITRO WHEN ISOLATED SALIVARY GLANDS OF THE LONE STAR TICK WERE EXPOSED TO DRUGS

Drug used in Experimental Phase (II)	n	Mean rate of secretion (nanoliters/minute)** Pre-Experimental Phase (I)	Experimental Phase (II)	$\Delta_{I \to II}$	P*	Post-Experimental Phase (III)
Control	4	71 ± 28	1 ± 1	(-71)		49 ± 32
Cyclic AMP (10^{-2}M)	4	80 ± 43	124 ± 42	(+44)	< 0.001	72 ± 53
Theophylline (10^{-2}M)	4	48 ± 16	57 ± 11	(+11)	< 0.001	115 ± 48
Pilocarpine (10^{-5}M)	3	36 ± 14	2 ± 2	(-34)	NS	91 ± 47
Pilocarpine (10^{-3}M)	3	43 ± 22	1 ± 1	(-42)	NS	55 ± 39
Noradrenaline (10^{-5}M)	4	58 ± 34	191 ± 60	(+133)	< 0.001	72 ± 32
Noradrenaline (10^{-3}M)	4	82 ± 27	194 ± 31	(+112)	< 0.001	76 ± 28
Glutamate (10^{-5}M)	3	104 ± 21	4 ± 4	(-100)	NS	128 ± 70
Glutamate (10^{-3}M)	3	41 ± 14	3 ± 1	(-38)	NS	25 ± 3
5-HT (10^{-5}M)	4	34 ± 9	1 ± 1	(-33)	NS	28 ± 17
5-HT (10^{-3}M)	4	63 ± 23	1 ± 1	(-62)	NS	18 ± 11

*as compared to the control $\Delta_{I \to II}$, a t test was used to determine statistical significance of data at the $P < 0.05$ level

**data is expressed as the mean rate of secretion in each phase ± S.E.M.

Figure 2. Rates of Salivary Fluid Secretion After Additions of Theophylline, Cyclic AMP and Dibutyryl Cyclic AMP With the Open Arrow Indicating Removal of the Cyclic AMP Analogue and Addition of Adrenaline-Saline; Theophylline and Cyclic AMP Were Replaced by Adrenaline-Saline After 32 Minutes (Closed Arrow). Results Indicate the Average of Three Trials With Cyclic AMP and Dibutyryl Cyclic AMP and Four With Theophylline.


Rate of Secretion (nl/min.)
Time (min.)
Theophylline
10-2 M
Cyclic AMP
10-2 M
Dibutyryl Cyclic AMP
10-2 M
0
10
20
30
40
50
60
70

Effects of Potential Inhibitors

Insights into possible energy needs for fluid transport and possible mechanisms by which ions move across cells can be obtained by the use of potential inhibitors. As illustrated by Figure 3, adrenaline was present during all three experimental phases of the determinations and served as a control for the remaining results. The rate of fluid secretion increased to 150 nl/min during the first 30 minutes and maintained a constant rate for the next 15 minutes. After 48 minutes the rate declined to approximately 100 nl/min in the post-experimental phase.

2,4-dinitrophenol significantly ($P < 0.05$) slowed secretion in the experimental phase (Table II). Kaufman and Phillips (1973c) found that ouabain completely inhibited salivary secretion at a concentration of 10^{-6}M. However, using my experimental design, ouabain did not significantly inhibit secretion during the experimental phase.

To clarify these findings, the psychotomimetic drug harmaline was used. Harmaline behaves as an inhibitor of the $(Na^{+}+K^{+})$-ATPase system by competing with Na ions in the Na-dependent phosphorylation reaction (Canessa et al., 1973). At a concentration of 10^{-3}M this drug completely blocked fluid secretion (Table II). At a 10^{-5}M concentration of the drug, inhibition was apparent, but I was unable to demonstrate a significant reduction in the rate of secretion. Recovery of glandular function was substantial at both concentrations during the post-experimental phase.

Figure 3. Control for Potential Inhibitors of Salivary Fluid Secretion With Glands Exposed to Adrenaline-Saline Solutions in All Three Phases. Secretory Rates During the Experimental Phase Served as the Control for Testing Potential Inhibitory Drugs; (↑) Removal; (↓) Addition of Fresh Bathing Medium. Each Point Represents the Average of Four Separate Experiments.

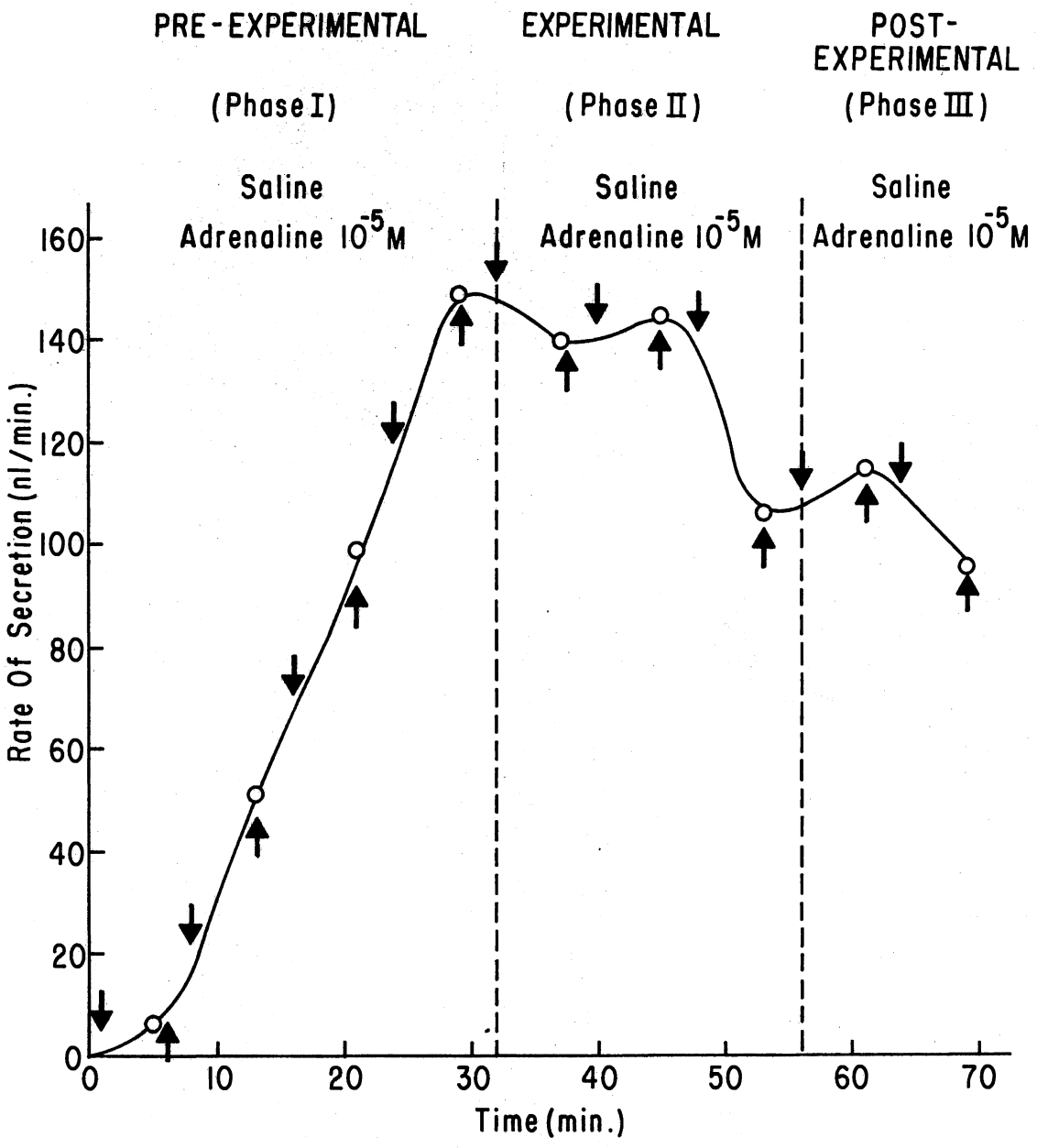

PRE-EXPERIMENTAL
(Phase I)
Saline
Adrenaline 10^{-5}M
EXPERIMENTAL
(Phase II)
Saline
Adrenaline 10^{-5}M
POST-EXPERIMENTAL
(Phase III)
Saline
Adrenaline 10^{-5}M
Rate Of Secretion (nl/min.)
0
20
40
60
80
100
120
140
160
0
10
20
30
40
50
60
70
Time (min.)

TABLE II

EFFECTS OF POTENTIAL INHIBITORS ON RATES OF FLUID SECRETION IN VITRO WHEN ISOLATED SALIVARY GLANDS OF THE LONE STAR TICK WERE EXPOSED TO DRUGS

Drug used in Experimental Phase (II)	n	Mean rate of secretion (nanoliters/minute)** Pre-Experimental Phase (I)	Experimental Phase (II)	I → II	P*	Post-Experimental Phase (III)
Adrenaline (control)	4	76 ± 40	131 ± 49	(+55)		105 ± 39
DNP (10^{-5}M)	4	48 ± 10	50 ± 12	(+2)	< 0.01	61 ± 10
DNP (10^{-3}M)	4	45 ± 16	41 ± 16	(-4)	< 0.01	2 ± 1
Ouabain (10^{-5}M)	4	58 ± 6	113 ± 37	(+55)	NS	60 ± 34
Ouabain (10^{-3}M)	3	22 ± 11	45 ± 23	(+23)	NS	1 ± 1
Harmaline (10^{-5}M)	4	62 ± 30	76 ± 44	(+14)	NS	113 ± 70
Harmaline (10^{-3}M)	4	49 ± 17	3 ± 1	(-46)	< 0.001	34 ± 13

*as compared to the control $_{I \rightarrow II}$, a t test was used to determine statistical significance of data at the $P < 0.05$ level

**data is expressed as the mean rate of secretion in each phase ± S.E.M.

CHAPTER V

DISCUSSION

The results agree with the findings of Kaufman and Phillips (1973b) in that catecholamines stimulate tick salivary fluid secretion. The results are also consistent with data where catecholamines stimulated chloride uptake into lone star tick salivary gland cells (Sauer et al., 1974). The latter findings were cited as evidence in support of the standing gradient hypothesis (Diamond and Bossert, 1967; 1968) to help explain the mechanisms of salivary fluid secretion.

The major difference and finding of present results in contrast to those published by Kaufman and Phillips (1973b) concerns the stimulatory effect of cyclic AMP and the phosphodiesterase inhibitor theophylline. The latter drug's effect was not investigated by Kaufman and Phillips. The glands of A. americanum secreted in the presence of cyclic AMP and theophylline, but only after pre-stimulation with adrenaline. I, as Kaufman and Phillips (1973b), was unable to achieve glandular secretion if cyclic AMP was administered to the glands before adrenaline administration. However, both cyclic AMP and theophylline alone are potent stimulants of chloride uptake into A. americanum salivary gland cells (Sauer et al., 1974). The question of importance is by what means does pre-incubation and stimulation with adrenaline render the glands susceptible to stimulation by cyclic AMP (and theophylline). One possible explanation is that adrenaline increases the cells permeability to

molecules such as cyclic AMP which is relatively impermeable (Berridge and Prince, 1972). However, the cyclic AMP derivative dibutyryl cyclic AMP, which reportedly mimics cyclic AMP and enters cells more readily Berridge and Prince, 1972; Drummond et al., 1974), was also ineffective as a lone stimulant of salivary secretion (Figure 2). Any assessment of the effectiveness of theophylline, or lack of, must take into account its low solubility in water (Goodman and Gilman, 1970). It is also well to keep in mind the effects of 5-HT and cyclic AMP on salivary secretion in Calliphora (Berridge, 1970; Berridge and Prince, 1972). The authors found differences in the mode of action of these two drugs even though both stimulate fluid secretion. They also implicated calcium as an important intracellular regulator. What role calcium might play in tick salivary fluid secretion awaits further investigation. Also of interest is the tendency for the dibutyryl cyclic AMP to retard glandular recovery when adrenaline was added to the bathing medium (Figure 2). Further experimentation is needed in regards to this, however.

Another interesting aspect of the present results was the effect noted after additions of ouabain and harmaline during adrenaline stimulation. Both chemicals have been shown to inhibit $(Na^{+}+K)$-ATPase systems, but by different mechanisms (Canessa et al., 1973). Considerable evidence indicates that ouabain inhibits ATPase by binding to the phosphorylated form of the enzyme (Charnock and Potter, 1969; Sen et al., 1969). Harmaline however, blocks phosphorylation of the enzyme by competing with Na ions, which are essential for this reaction to occur (Canessa et al., 1973). Harmaline was an effective inhibitor of fluid secretion at concentrations of 10^{-3}M during the experimental phase even though inhibition was apparent, I was unable to demonstrate a signifi-

cant inhibition with 10^{-3}M ouabain during the experimental phase. However, during the post-experimental phase, some secretion was restored in the harmaline treated glands but not in the ouabain treated glands (Table II). One might speculate that the delayed recovery by ouabain (10^{-3}M) treated glands could be due to a more involved inhibitor-enzyme interaction; whereas harmaline seems to displace Na ions by competitive inhibition and does not seemingly require chemical bonding (Canessa et al., 1973). It is also worth noting that Kaufman and Phillips (1973b) completely inhibited secretion after soaking the glands of D. andersoni for 80 minutes in a medium containing 10^{-6}M ouabain. It may be that ouabain and to a lesser extent, harmaline, are drugs that enter the cells of the glands slowly and the $(Na^{+}+K^{+})$-ATPase system, if present, is located on the apical side of the fluid secreting cells. The presence of an energy requirement for fluid transport is supported by the reduction in secretion after addition of DNP to the bathing medium.

I, as Kaufman and Phillips (1973b), was unable to stimulate glands to secrete with pilocarpine in the bathing medium. Pilocarpine has been used by a number of workers to stimulate salivary secretion in vivo (Barker et al., 1973; Purnell et al., 1969; Tatchell, 1967). The question of interest, therefore, regarding pilocarpine is its site of action. We have preliminary information (Eikenbary[1]) indicating that pilocarpine affects the electrical potential across the tick gut epithelium. Whether the gut is the initial site of action of pilocarpine

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[1]Eikenbary, P., Personal Communication, Department of Entomology, Oklahoma State University, Stillwater, Oklahoma, 1974.

after *in vivo* administration in the tick is still speculative, however.

My results on the effectiveness, or lack of, of 5-HT and glutamate are consistent with the findings of Kaufman and Phillips (1973b) and the ineffectiveness of these drugs in stimulating chloride uptake into salivary gland cells of the lone star tick (Sauer et al., 1974).

CHAPTER VI

SUMMARY AND CONCLUSIONS

This investigation was designed to determine possible mechanisms and control of fluid secretion in salivary glands of the feeding female lone star tick, Amblyomma americanum (L.) using an in vitro technique. Various drugs and/or inhibitors were incorporated into the bathing medium surrounding isolated glands and the effect on fluid secretion was measured by recording changes in the rate of fluid secreted.

Adrenaline, noradrenaline, cyclic AMP and theophylline were effective stimulants of in vitro salivary fluid secretion in glands. The later two drugs were effective only after the glands were pre-stimulated with adrenaline. Pilocarpine nitrate, 5-hydroxytryptamine and glutamate were ineffective. 2,4-dinitrophenol significantly inhibited the ability of adrenaline to stimulate secretion.

Inhibitors of $(Na^{+}+K^{+})$-ATPase systems, ouabain and harmaline were tested. Harmaline at a concentration of 10^{-3}M immediately inhibited adrenaline's effectiveness. At 10^{-3}M concentration ouabain was not immediately effective, but following glandular exposure to the adrenaline-ouabain combination, recovery of secretory competence could not be attained. I was unable to demonstrate any significant inhibitory effect by these two drugs at 10^{-5}M concentration. Inhibition of salivation by DNP suggests energy is required for movement of fluid across glandular cells.

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VITA

Glen Ray Needham

Candidate for the Degree

Master of Science

Thesis: IN VITRO STUDIES ON CONTROL OF FLUID SECRETION BY ISOLATED SALIVARY GLANDS OF THE LONE STAR TICK AMBLYOMMA AMERICANUM (LINNEAUS)

Major Field: Entomology

Biographical:

Personal Data: Born in Prowers County, Lamar, Colorado, December 25, 1951, the son of Robert and Dollie Needham.

Education: Graduated from Beaver High School, Beaver, Oklahoma, in May, 1969; received the Bachelor of Science degree from Southwestern Oklahoma State University, Weatherford, Oklahoma, with a major in Biology and a minor in Chemistry, July, 1973; completed requirements for the Master of Science degree at Oklahoma State University in May, 1975.

Professional Experiences: Research assistant, Department of Entomology, Oklahoma State University, Stillwater, Oklahoma, January, 1974 to May, 1975.

Societies: Entomological Society of America; Sigma Xi.

Honors: Received National Science Foundation Summer Research Fellowship, Department of Entomology, Oklahoma State University, June to August, 1974.