Protein Phosphorylation and Control of Tick Salivary Gland Function

J.R. SAUER, J.L. McSWAIN, J.S. TUCKER, K.S. SHELBY, J.P. WILLIAMS and R.C. ESSENBERG*

Departments of Entomology and Biochemistry* Oklahoma State University, Stillwater, Oklahoma 74078 (U.S.A.)

(Accepted 8 December 1988)

ABSTRACT


Tick salivary glands are controlled by nerves, dopamine being a neurotransmitter at the neuro-effector junction. Dopamine and cyclic AMP (cAMP) stimulate fluid secretion by isolated salivary glands. Dopamine activates an adenylate cyclase to increase intracellular cAMP within the female salivary glands. Phosphoproteins whose levels of phosphate are affected by cAMP-dependent protein kinase have been identified in subcellular fractions. Protein(s) phosphorylated by cAMP appears to activate protein phosphatase in the salivary glands.

Another phosphorylation pathway appears to act through protein kinase C because of an ability of phorbol esters (known activators of protein kinase C) to stimulate the phosphorylation of proteins, and an ability of a peptide factor in tick brain to metabolize salivary-gland phosphoinositides, an event that often precedes activation of protein kinase C. Because cAMP modulates brain-factor-stimulated formation of inositol phosphates (products of phosphoinositide breakdown) an interrelationship between the two pathways seems likely.

Evidence of regulatory processes, including protein phosphorylation/dephosphorylation reactions, will provide a basis for helping assess the physiological significance of secretory products and the role of the salivary glands in disease transmission.

INTRODUCTION

Interest in the morphology, physiology, and control of tick salivary glands relates to their role in tick feeding and their importance in enabling the tick to vector pathogens. Ixodid tick female salivary glands are morphologically and physiologically complex. The morphology of tick salivary glands has been reviewed in detail by Fawcett et al. (1986). Secretory products include: cement to help anchor the mouthparts to the host; anticoagulants, in some species; agents that appear to increase the flow of the host’s blood to the attachment site; substances that induce the release of mediators of inflammation in the
host; and enzymes whose functions have yet to be defined (Fawcett et al., 1986).
Anti-hemostatic, anti-inflammatory and anti-histaminic substances, which may assist the tick in feeding by counteracting host defense mechanisms, have been found in the saliva of some species (Kemp et al., 1982; Ribeiro et al., 1985; Ribeiro and Spielman, 1986). Salivary products are also antigenic and induce tick resistance in the host (Brown and Askenase, 1986; Gill et al., 1986).

A notable feature of female tick salivary glands is their ability to excrete copious amounts of fluid back to the host to facilitate concentration of the bloodmeal. Ixodid females are relatively slow feeders: e.g., *Amblyomma americanum* requires 9–15 days to complete feeding. The feeding cycle consists of a slow phase and a rapid phase, the latter occurring during the last 12–24 h.

Female *A. americanum* increase in mass from approximately 4 mg to about 650 mg. As remarkable as this increase is, it greatly underestimates the total amount of host blood ingested. Approximately 1/3 to 1/2 of all tissue fluid ingested is eliminated by the female back to the host via the salivary glands in the form of excess water and ions to help concentrate the bloodmeal (Sauer and Hair, 1972; Koch and Sauer, 1984). Unlike the situation in insects, very little fluid is excreted externally through the Malpighian tubules.

To understand the normal process of fluid secretion, it is imperative that one demonstrate the existence of metabolic pathways regulating synthesis and/or secretion of secretory products. The salivary glands are richly innervated and, based upon pharmacological studies, dopamine is a neurotransmitter at the neuroeffector junction controlling secretion (Kaufman, 1976). Dopamine stimulates fluid secretion by isolated salivary glands when Ca²⁺ is present in the external medium (Kaufman, 1976; Needham and Sauer, 1979) and dopamine also causes salivation when injected into whole ticks (Hsu and Sauer, 1975). Various agonists are known to stimulate adenylate cyclase receptors on the plasma membrane. Adenylate cyclase increases the level of the ‘second messenger’ cAMP which activates a cAMP-dependent protein kinase resulting in phosphorylated proteins. Phosphorylation of proteins results in changes in the proteins that leads to some physiological response. Because dopamine activates a plasma-membrane-associated adenylate cyclase to increase cAMP from ATP within the salivary glands (Schmidt et al., 1981; 1982, fig. 1) and because exogenous cAMP stimulates secretion by isolated salivary glands (Needham and Sauer, 1979), we have been investigating mechanisms of signal transduction and ‘second-messenger’ biochemistry to determine how this pathway controls secretion during tick feeding. It should be stressed that all studies have been performed on whole-gland preparations, isolated tissue fractions or partially purified proteins and it is still not possible to assign metabolic events to specific cells within the morphologically complex tissue, nor for that matter outline a complete sequential mechanism of how a salivary product is synthesized and/or secreted.