Amiloride high- and low-sensitivity, as well as insensitive sites in the blowfly: implications for sugar, water and anion taste reception mechanisms

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Abstract—Among chemoreceptor cells of the blowfly (Protophormia terraenovae), the so-called ‘sugar’ and ‘water’ cells respond to bovine serum albumin (BSA) and L-alanine (the C-terminal amino acid of the BSA molecule) with the highest spike frequency. In both cell types the response to either BSA or L-alanine was unaffected by 0.1 mM amiloride. However, 0.5 mM amiloride decreased the response of the ‘water’ cell to both BSA and L-alanine significantly, and similarly inhibited the response of the ‘sugar’ cell to L-alanine, but in contrast to the ‘water’ cell, 0.5 mM amiloride did not affect the ‘sugar’ cell response to BSA. The ‘sugar’ cell responses to both BSA and L-alanine were unaffected by the putative cell second messengers, cAMP and cGMP. The so-called ‘anion’ cell unexpectedly gave responses to BSA and L-alanine that were enhanced by amiloride at both concentrations. However, like the ‘sugar’ cell, the ‘anion’ cell was also unaffected by cAMP and cGMP. We conclude that the ‘sugar’ cell must have at least three types of receptor sites: the previously described ‘F’ (amiloride sensitive) and the ‘P’ (amiloride insensitive) sites, and a low-sensitivity ‘T’ site that mediates, at least in part, the response to BSA and L-alanine. The effects of amiloride on the responses of the ‘water’ and ‘anion’ cells are more difficult to interpret because fundamental information on their chemoreception mechanisms is still lacking.

Keywords: ‘Sugar’ receptor; ‘water’ receptor; ‘anion’ receptor; blowflies; amiloride; albumin; amino acids.

1. INTRODUCTION

Amiloride is known to affect salty and sweet transduction processes in a variety of vertebrate species, with the former quality being the most documented (Desor and Finn, 1989; Schiffman et al., 1990a, b; Cummings et al., 1993; Ossebaard and Smith, 1994). An amiloride-sensitive cation conductance has been found also in...
insects; however, unlike the case of most vertebrates, this site is apparently involved in the sugar reception mechanism only (Jenkins and Tompkins, 1990; Liscia et al., 1997). In particular, in the labellar chemoreceptors of blowflies (*Protophormia terraenovae*), this amiloride-sensitive cation channel is thought to mediate fructose sweet transduction, while sucrose stimulation seems to be unaffected by amiloride (Liscia et al., 1997).

In blowflies, four chemoreceptor cells are housed in each labellar chemosensillum and are named according to their best stimulus as ‘water’, ‘salt’, ‘sugar’ and ‘anion’ (5th) cells (Evans and Mellon, 1962b; Dethier and Hanson, 1968; Rees, 1970; Rees, 1972; Dethier, 1976; Dethier, 1987; Schnuch and Hansen, 1990). The spikes recorded extracellularly from each of the four cells consistently differ in amplitude and shape, and can be separated from one another (Dethier and Hanson, 1968; Dethier, 1976; Schnuch and Hansen, 1990; Schnuch and Hansen, 1992). Indeed, a rigid response specificity has not been documented and each cell may also be responsive to other chemicals, such as amino acids (Dethier and Hanson, 1968; Dethier, 1976; Schnuch and Hansen, 1990; Schnuch and Hansen, 1992). For instance, the ‘sugar’ labellar chemoreceptor cell is also stimulated by bovine serum albumin (BSA) and its C-terminal amino acid (L-alanine), that may be considered as representative of proteinaceous food sources for blowflies (Liscia et al., 1995a, b). A fourth site specific for protein molecules has indeed been proposed in the ‘sugar’ cell of blowflies (Liscia et al., 1995a, b) in addition to the three receptor sites previously documented, i.e. a pyranose ‘P’ site, a furanose ‘F’ site and an aliphatic carboxylate ‘T’ site (Shimada et al., 1974, 1985; Shimada and Isono, 1978; Shimada and Tanimura, 1981; Shimada, 1987).

On the basis of the above considerations, we have studied the effects of amiloride on the responses to BSA and L-alanine (its C-terminal amino acid), with the aim of gaining further evidence on taste chemoreception mechanism(s) in blowflies, and in particular on the receptor site types in the ‘sugar’ cell involved in the chemoreception of amino acids and proteins. We also tested the effects of cAMP and cGMP, as in blowflies these cyclic nucleotides seem to have a role in sweet transduction (Liscia et al., 1987, 1989a–c; Amakawa et al., 1992) and, on the other hand, adenylate cyclase, the Na\(^+\) pump and various types of cyclic nucleotide-gated channels have been proposed as possible targets of the action of amiloride (for a review, see Lindemann, 1996).

2. MATERIALS AND METHODS

Experiments were performed on three to five of the ‘largest’ labellar chemosensilla (Wilczek, 1967) of each of 15–20 adult female blowflies, *P. terraenovae* (3–5 days old). The insects came from our colony maintained under standard conditions. The spike activity in response to the stimuli presented to each sensillum was recorded by means of the tip-recording method of Hodgson et al. (1955): the reference electrode (a silver/AgCl wire) was inserted in the foramen magnum of an isolated head; the