Journal of Insect Physiology 56 (2010) 991–997

Contents lists available at ScienceDirect

Journal of Insect Physiology

journal homepage: www.elsevier.com/locate/jinsphys

# Locusts as model organisms in which to study immunogen-induced anorectic behaviour

Graham Goldsworthy *

*Biology Department, School of Biological and Chemical Sciences, Birkbeck College, University of London, Malet Street, London W1CE 7HX, UK*

ARTICLE INFO

*Article history:*
Received 26 February 2010
Received in revised form 13 April 2010
Accepted 14 April 2010

*Keywords:*
AKH
Anorexia
Feeding behaviour
Laminarin
*Locusta*
LPS
Mianserin
Serotonin

ABSTRACT

When injected into adult or nymphal *Locusta* that have been deprived of food for 2 h, immunogens such as laminarin and bacterial LPS can induce an almost immediate dose-dependent state of anorexia for at least 1 h. Such anorexia is a component of a medley of physiological and behavioural changes called collectively 'sickness behaviour' that occurs in a wide range of animals in response to infection or immune challenge. Sub-optimal amounts of injected laminarin allow some locusts to feed, but with a longer latency than in controls, although the length of the first meal is unaffected. The feeding behaviour of fifth instar nymphs is more sensitive to laminarin than that of adults, but both stages respond to amounts of immunogen that are lower than those required to activate the phenoloxidase cascade. Injection of adipokinetic hormone (AKH) before the period of food deprivation prevents the anorexigenic action of the laminarin in adults but not in nymphs. It is argued that the effect of the AKH may be indirect, through its lipid-mobilising action. The insecticide pymetrozine increases the latency to feed but also reduces the length of the first meal, and its anorexigenic activity is not affected by injection of AKH. The present data support the concept that laminarin-induced anorexia involves a central lack of motivation to eat, rather than a 'stop eating' signal. Others have shown that the mechanism of action of pymetrozine involves the serotonergic system and can be blocked by mianserin, so it is intriguing that in the present study injection of mianserin prior to that of laminarin modulates the anorexigenic effect of the immunogen. This suggests that biogenic amines are involved in the control of appetitive behaviour in locusts, as they are in vertebrates. The possible usefulness of the locust model in studying sickness-induced anorexia is discussed briefly.



## 1. Introduction

Recent studies have suggested that locusts are good models in which to study the pathogenesis of microorganisms such as the bacterium *Escherichia coli* (Khan and Goldsworthy, 2007; Mokri-Moayyed et al., 2008) and the protozoan *Acanthamoeba* (Mortazavi et al., 2009), especially in the ability of these organisms to invade the central nervous system. In the present study, the locust is used as a model again, but to study another aspect of infection, sickness behaviour. The general physiology and metabolism of animals changes dramatically following infection, with sick individuals having little motivation to eat and becoming listless. These behavioural changes are described as 'sickness behaviour' and are thought to be a manifestation of a central motivational state designed to promote recovery (Kelley et al., 2003) by diverting energy away from normal activities to overcome infection, perhaps by depriving the pathogen of nutrients (Exton, 1997).

Sickness behaviour occurs in a wide range of animals, and can include immunological, neuroendocrine, metabolic and behavioural changes. Two common examples of sickness behaviour which can be demonstrated in insects are anorexia and fever (for review, see Adamo, 2008). Locusts thermoregulate behaviourally in order to maintain an elevated temperature when infected with a fungal pathogen, suggesting that 'behavioural fever' is a feature of the immune system (Bundey et al., 2003). In this study, the effects of an immune challenge on the feeding behaviour of locusts is examined for the first time by injecting immunogens such as bacterial lipopolysaccharide (LPS) or the (predominantly) β-1,3-glucan, laminarin, to mimic respectively the initial stage of a bacterial or fungal infection (see Goldsworthy et al., 2002). In addition, because adipokinetic hormone (AKH) enhances the activation of phenoloxidase (Goldsworthy et al., 2002) and the nodulation (Goldsworthy et al., 2003a,c) responses to immunogens in locusts, possible effects on feeding behaviour of co-injection of AKH at the same time as immune challenge are also investigated. Finally, a comparison is made between anorectic behaviour induced in locusts by an immunogen, and that induced by the pesticide pymetrozine (see Kaufmann et al., 2004) to

* Tel.: +44 020 8441 0951.
*E-mail address:* gjgoldsworthy@googlemail.com.

0022-1910/$ – see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.jinsphys.2010.04.005

investigate the possible involvement of biogenic amines in effecting anorexia.

## 2. Materials and methods

### 2.1. Insects

Adults or fifth instar nymphs of *Locusta migratoria* (L.) were used in the assays described in this study. The insects were bred in a colony maintained at Birkbeck under crowded conditions at 30 °C in a 12:12 light/dark cycle, and fed daily with rolled bran and fresh grass supplemented with wheat seedlings. Seedlings of organic winter wheat were grown on damp tissue paper and used as a food source for the locusts when the leaves were 12–15 cm tall. No account was taken of whether the nymphs used in experiments were male or female, but all experiments with nymphs were conducted 2–4 days after the moult from the IVth to the Vth stadium. With adult locusts, only males between 12 and 25 days old were used to avoid complications caused by variations in feeding behaviour in females at different stages of egg development.

### 2.2. General animal procedures

The assay of feeding behaviour was a modification of that described by Goldsworthy et al. (2003b), and was undertaken in a small room separate from the main colony. This room was brightly lit, quiet, and maintained at 30 °C. Prior to the commencement of each assay, hoppers were given access to fresh wheat seedlings, and observed until an individual had eaten a meal, when it was grouped with others that had similarly been observed to eat a meal in a fresh cage without food or water. Such a group of 20–30 locusts was left for 2 h before the feeding assays.

Injections were administered using plastic pipette tips each of which had a short stainless steel needle held in its bore by friction. The injection was made into the haemocoel by inserting the needle through the intersegmental membrane between two abdominal terga, and expelling the injectate using an automatic pipette set to deliver 20 μl. After injection, the abdomen was gently extended and compressed longitudinally several times to ensure rapid mixing of the injectate with haemolymph. In some experiments, two separate injections were administered approximately 10 min apart. Although clumsy handling of locusts, especially by inexperienced students, can affect feeding behaviour, preliminary studies showed that in the hands of experienced workers Ringer-injected locusts behaved in a similar manner to non-injected locusts in the feeding assay described here (data not shown). Nevertheless, in the experiments described in this study, care was taken to ensure that experimental and control locusts were each subjected to the same number of injections.

After injection(s), locusts were placed together in a small holding cage for 10 min until the beginning of the assay. To start the feeding assay, locusts were placed individually in transparent rectangular plastic boxes (17.5 cm × 10.5 cm × 6 cm, height × width × depth) together with fresh intact wheat seedlings oriented so that they could be used as a vertical perch. Groups of 12 locusts (6 controls and 6 experimental) were set up in this way and observed for 1 h, during which whether each individual was eating or not was noted at intervals of 1 min. Care was taken to distinguish between eating (where the mandibles could be seen to be removing pieces of leaf) and palpating (where the sensory palps were used to test the quality of the potential food source). For the purposes of this study, a meal was defined as any feeding episode that comprised two or more consecutive minutes of eating as described above.

### 2.3. Preparation of solutions for injection

All chemicals were purchased from Sigma Chemical Company. Two immunogens were used: laminarin (a β-1,3-glucan from seaweed that is fairly typical of the glucans found in fungal cell walls) and bacterial lipopolysaccharide (LPS or endotoxin from *E. coli* in this instance), which is a major component of the cell wall of Gram negative bacteria. Stock solutions of LPS (5 mg/ml) or laminarin (2 mg/ml) made up in sterile locust Ringer (168 mM NaCl; 6.4 mM KCl; 3.6 mM $MgCl_2$; 6 mM $NaH_2PO_4$; 2.1 mM $NaHCO_3$; 20 mM Hepes; 2.1 mM $CaCl_2$) were stored frozen at −20 °C, and then thawed and diluted appropriately with fresh Ringer before each assay. A stock solution of locust adipokinetic hormone 1 (hereafter called AKH) was made up in 80% methanol (10 pmol/μl), stored at −20 °C until required and then diluted 20-fold with sterile Ringer before injection. Stock solutions of mianserin ($10^{-3}$ M) or pymetrozine (5 mg/ml) were made up in dimethylsulphoxide (DMSO), and diluted 10 and 5-fold respectively (at least) in sterile Ringer before use. When mianserin or pymetrozine were being tested, the control locusts were injected with locust Ringer containing 20% DMSO.

### 2.4. Data analysis

From the records kept during the 1 h of each feeding assay, the delay (if any) before each individual locusts started to eat (hereafter called the latency to feed) and the length of the first meal were recorded. Because in some experiments, individual locusts did not eat at all during the 1 h assay, the percentage of locusts not eating overall was also noted. Data for latency to feed and meal length were analysed through a series of unpaired *t*-tests. When variance of the mean is indicated on figures the vertical bars represent ±1SE.

## 3. Results

### 3.1. Injection of laminarin

Fig. 1 shows that injection of as little as 10 μg of laminarin into adult male locusts that had been deprived of food for 2 h prevented the locusts from feeding during the 1 h test period. These laminarin-injected adults tended to perch on the seedlings and remain static for long periods, seldom even palpating the blades of wheat. Ringer-injected control locusts that had been deprived of food for 2 h, however, approached a wheat leaf quickly and started to eat within *c.* 2 min (Fig. 1A and B): on average these adult control locusts ate a first meal for *c.* 4.5 min and had two more feeding episodes during the assay period (Fig. 1A), but only the first meal taken in each assay is considered in this study. When only 5 μg of laminarin were injected after the period of food deprivation *c.* 85% of the adult locusts did eat, but with a significantly ($P < .05$) increased latency compared with Ringer-injected locusts (Fig. 1B), whereas the length of the first meal was similar in each group.

When the above protocol was used with Vth instar nymphs, similar results to those with adults were obtained (Fig. 2A and B); laminarin-injected nymphs perched and remained very still on the seedlings, not palpating or eating the blades of wheat. However, the feeding behaviour of nymphs appeared more sensitive to the injected laminarin than that of adults, with as little as 2 μg of laminarin inducing anorexia during the 1 h assay: although the body weights of nymphs and adults may differ by a factor of 2 or 3, the blood volumes of nymphs at mid-instar and mature adult males are very similar (Hill and Goldsworthy, 1969). When only 0.5 μg of laminarin was injected after the period of food deprivation *c.* 75% of the nymphs ate, but with a significant ($P < .001$) increase in latency, although the length of the first (and