General and Comparative Endocrinology 166 (2010) 222–233

Contents lists available at ScienceDirect

General and Comparative Endocrinology

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

Review

In search for a common denominator for the diverse functions of arthropod corazonin: A role in the physiology of stress?

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ARTICLE INFO

Article history:
Received 16 July 2009
Accepted 4 September 2009
Available online 11 September 2009

Keywords:
Myotropin
Rhythms
Insect
Stress
Adrenalin
Insect hormones
Juvenile hormone
Ecdysone
Neuropeptides
Clock
PDF
Corazonin

ABSTRACT

Corazonin (Crz) is an 11 amino acid C-terminally amidated neuropeptide that has been identified in most arthropods examined with the notable exception of beetles and an aphid. The Crz-receptor shares sequence similarity to the GnRH-AKH receptor family thus suggesting an ancestral function related to the control of reproduction and metabolism. In 1989, Crz was purified and identified as a potent cardio-accelerating agent in cockroaches (hence the Crz name based on "corazon", the Spanish word for "heart"). Since the initial assignment as a cardioacceleratory peptide, additional functions have been discovered, ranging from pigment migration in the integument of crustaceans and in the eye of locusts, melanization of the locust cuticle, ecdysis initiation and in various aspects of gregarization in locusts. The high degree of structural conservation of Crz, its well-conserved (immuno)-localization, mainly in specific neurosecretory cells in the pars lateralis, and its many functions, suggest that Crz is vital. Yet, Crz-deficient insects develop normally. Upon reexamining all known effects of Crz, a hypothesis was developed that the evolutionary ancient function of Crz may have been "to prepare animals for coping with the environmental stressors of the day". This function would then complement the role of pigment-dispersing factor (PDF), the prime hormonal effector of the clock, which is thought "to set a coping mechanism for the night".

1. Introduction

The neuropeptide corazonin (Crz) was named based on its functional ability to accelerate, at very low concentrations, heartbeat in the cockroach *Periplaneta americana* in a semi-*in vivo* assay (Veenstra, 1989). This finding suggested an important role in controlling heartbeat in insects. However, it soon became clear that one should not readily make generalizations as the effects in *P. americana* seemed to be the exception rather than the rule in Blattaria (Predel et al., 1994, 2001) and other insects. Hence, Predel et al. (2001) concluded that modulation of heartbeat in *Periplaneta* by Crz was possibly non-essential suggesting that the true function(s) of Crz in arthropods remained to be discovered.

With respect to the physiology of the insect heart, the second surprising observation was that Crz was much less active *in vivo* than *in vitro*. Slama (2004) reported that in *Manduca sexta*, dosages below 10^{-7} M were completely ineffective. Higher dosages (10^{-6} M final concentration of Crz in the body) caused an almost immediate, adrenalin-like enhancement of the anterograde heartbeat. During the short acute phase, heartbeat increased on average from 10.5 to 24 pulses per min. plateauing at 2.5 min after injection. This brief tachycardia induced by high dosage was the first (unintentional) demonstration that Crz may serve a role in the physiology of stress. Later, Slama et al. (2006) became even more critical of the possible *in vivo* hormonal role of Crz and other peptidic cardiostimulants. They classified the potent cardiostimulatory activity of Crz and other peptides as an artifact as this effect was not found in animals that were decapitated before or after peptide injection.

A very different function for Crz was discovered by Tawfik et al. (1999) who searched for hormones that promoted the gregarious phase of locusts. Gregarious *Locusta migratoria* and *Schistocerca gregaria* deposit more melanin in their cuticle than do solitarious animals. To their surprise, they found that the "dark pigmentotropin" was an isoform of Crz, namely [His^7]-corazonin, as compared to [Arg^7]-corazonin of *Periplaneta*.

The link to a possible role in the physiology of stress was not made at that time.

In our laboratory, in the beginning of 2009, some 20 last instar nymphs of the desert locust *S. gregaria* had been caged in a small plastic rearing cage (8 × 8 × 15 cm) in preparation of an injection experiment. The experiment was cancelled but the animals were left in the small cage, clearly in conditions of overcrowding, and fed daily. Surprisingly, the cuticle of the animals started to turn black after a few days and became very black towards the end of the instar. The black color persisted into the adult cuticle (Fig. 1).

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0016-6480/$ - see front matter
doi:10.1016/j.ygcen.2009.09.004

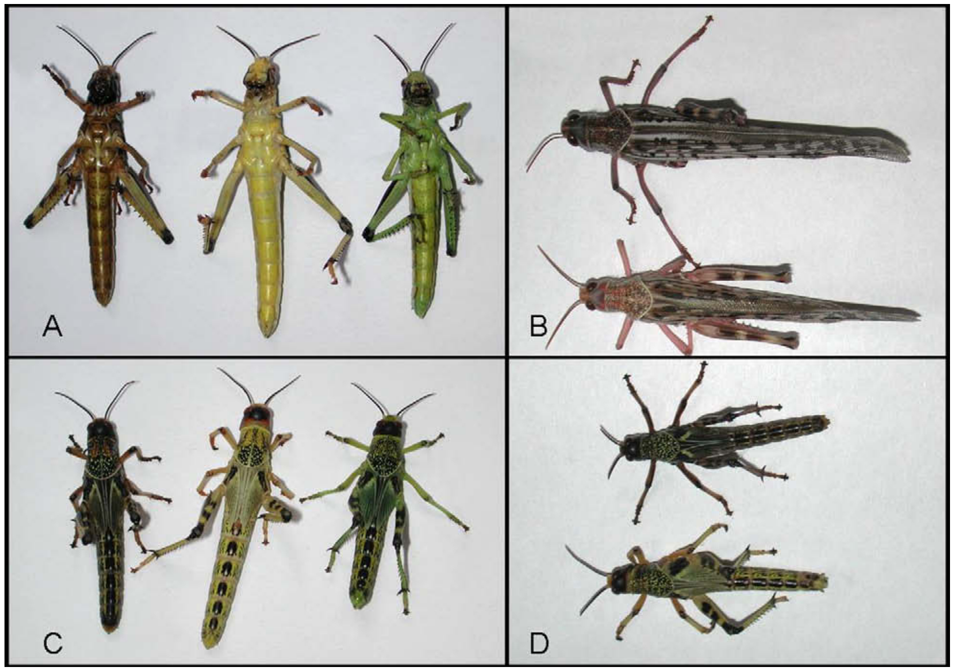

Fig. 1. Cuticular melanization in *Schistocerca gregaria* in function of population density. Fifth instar nymphs taken from a big breeding cage (40 × 32 × 48 cm) with gregarious animals were either isolated or grouped (20 animals) in a small (8 × 8 × 15 cm) plastic rearing box) within 24 h after the 4L–5L nympal molt. Towards the end of the 5th instar, the isolated animals turned much greener (right animal in panels A and C), while the overcrowded ones turned much blacker (left animal in panels A and C) as compared to a regular animal bred in normal gregarious conditions (middle animal in panels A and C. (A) ventral view; (C) dorsal view. (B and D) show two animals that were monitored from the 5th instar (D) into the adult stage, illustrating that the blacker cuticular color of the overcrowded nymph (the upper animal in both panels B and D), as compared to a normal gregarious animal (the lower animals in panels B and D) persists into the adult stage. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

This observation stimulated us to re-examine all published data on the physiological and behavioral effects of Crz in the conceptual framework of a (central) role in the physiology of stress. This challenged us with several questions such as: ''What is stress?'', ''Do insects have stress?'', ''Where is the borderline between normal physiology and stress physiology?'', and ''Is there a link with the diurnal clock system?'' These are questions to which there are no easy answers, as stress in invertebrates is a very poorly explored field.

The term ''stress'' was originally introduced by the endocrinologist Hans Selye (1936) in reference to the failure of human or animal body reactions to unpleasant and harmful stimuli. To date, this definition of stress has also been applied to the physiology of stress in many non-vertebrate model systems such as yeasts, plants, and invertebrates.

Organisms engage in alarm-, resistance- and eventual exhaustion reactions. All these aspects of ''stress'' are tested, when an organism's living conditions (continue to) deviate too much from average values. Well documented stressors are extreme temperatures (too cold and too warm) and humidity conditions (too dry or too wet), light intensity, oxidative stress, food shortage, poisons, the presence of all sorts of enemies, excessive population density, and/or a lower position in the dominance hierarchy when present. The intensity of some stressors changes according to a diurnal- and seasonal rhythm. Hence the signaling systems of the clock and of stress are likely to be closely intertwined.

The borderline between eustress and distress is difficult to draw. What is experienced by some individuals as ''easily bearable'' may be ''unbearable'' for others. In fact any signaling molecule can be regarded as a mild stressor. Indeed they all influence the conformation of their receptor or receiver. When the effects are (very) weak in the context of the whole body, they may not be experienced as stressful.

Like vertebrates, insects have an autonomous and non-autonomous nervous system. In vertebrates, the autonomous nervous system is of primary importance in coordinating the actions of the sympathetic and parasympathetic systems in fight- and flight-reactions. The role of hormones, in particular of adrenalin, in stress physiology is much better documented in higher vertebrates than in any invertebrate. Adrenalin, first described in insects by Ostlund (1954), is now known to be a very minor member of the biogenic amine family in insects. Serotonin, dopamine and octopamine are much more important members. In addition to biogenic amines neuropeptides may also play a role in stress physiology. Recently, Kodrik (2008) interpreted some lesser known functions of adipokinetic hormones (AKHs) that are not associated directly with flight activity in the perspective of stress. These included AKH-stimulated walking, diel fluctuations in AKH activities, and AKH's influence on the ability to cope with infections and toxins or other kinds of stressors. This work emphasized the importance of AKH neuropeptides in stress physiology.

This review addresses the question: ''Are Crz's apparently unrelated functions a consequence of functioning as a common factor in the initiation of physiological stress pathways?''

1.1. The original discovery of corazonin

At the moment that Jan Veenstra started his search for control agents of heartbeat in the cockroach *P. americana*, two cardioexcitatory octapeptides had already been identified, namely one belonging to the crustacean Red Pigment Concentrating Hormone (RPCH) family, and the other to the (AKH) family (Veenstra, 1989). Again in an extract of corpora cardiaca of *Periplaneta*, Veenstra (1989) identified a very potent third cardioexcitatory peptide, which he named ''corazonin'' after the Spanish word for ''heart'', ''corazon''. This undecapeptide stimulated the heartbeat at concen-