A Novel Receptor Capable of Monitoring Applied Pressure in the Abdomen of an Insect

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A pair of receptors, responding tonically to pressure applied internally, occurs on the ventral body wall of abdominal segments two to five in the adult of the blood-feeding insect, *Rhodnius prolixus*. These receptors are located in a region of the body wall subject to forces directly related to the size and movement of the stomach, an enlarged region of the midgut which stores the blood meal, and are therefore well suited for monitoring the degree of distension there. The initiation of many endocrinological processes in *Rhodnius* is known to be associated with the detection, over a period of several days, of abdominal distension, and these sensory receptors are capable of responding to abdominal distension for extended periods of time.

IN THE STUDY OF INSECT GROWTH AND reproduction, few insects have attained the status reserved for *Rhodnius prolixus*, a blood-feeding insect (sometimes referred to as the "kissing bug") that has been used for more than 50 years to study the control of growth, development, and reproduction (*1*). Its success as an experimental model arises in part from the fact that a single large blood meal sets in train a series of endocrinological events that culminates in moulting or egg production, depending on the stage of the life cycle. The degree of distension of the abdomen, caused by the ingestion of blood, is important in regulating hormone release: severing the abdominal nerves prevents the release of prothoracicotropic hormone (PTTH) (*2*); release of diuretic hormone is correlated with abdominal distension (*3*); the duration of egg production, which is governed by juvenile hormone (JH), is closely correlated with stomach size (*4*). As in many other insects (*5*), stretch receptors have been assumed to provide the essential link between the degree of abdominal distension and the regulated release of hormones.

Stretch receptors have been described in the abdomen of *Rhodnius* (*6*) and in the related blood feeder *Dipetalogaster maximum* (*7*). These receptors are on the dorsal (DIM) or the ventral intersegmental muscles (VIM), or both, and respond to stretch applied to these muscles. Because these receptors are rapidly adapting, they are not suited to monitor distension for several days, which is the length of time required for the release of PTTH and JH. In *Dipetalogaster* (*7*), a sustained electrical discharge was recorded from the abdominal nerve if the branch of that nerve innervating the medial side of the VIM was stretched. The electrical characteristics of the receptor giving rise to this discharge would enable the receptor to monitor long-term events, but the structure and location of this receptor was not identified.

Our studies of the electrical activity recorded extracellularly from the abdominal nerves of *Rhodnius* have revealed a nervous structure that may represent the unidentified tonically discharging mechanoreceptor described in *Dipetalogaster* (*7*). Interestingly, this receptor does not respond to separation of the intersegmental muscles as would be expected for abdominal stretch receptors monitoring expansion or stretching of the intersegmental membranes. Instead, this receptor responds best to pressure applied internally and increases its tonic rate of discharge as pressure is increased. This receptor is therefore well suited for monitoring the degree of distension of the stomach for long periods of time.

The abdomen of adult *Rhodnius* is supplied by abdominal nerves arising from the mesothoracic ganglion (*8*). Abdominal segments one to three receive innervation from nerves arising directly from the mesothoracic ganglion; segments four and five receive innervation from nerves that are branches of the large medial nerves innervating the organs in segment six. Each nerve or nerve branch that innervates segments two to five extends from the thorax, or the anterior region of the abdomen, to the lateral region of its corresponding segment. Anterior to the level of its abdominal segment, each nerve gives rise to two branches. These branches innervate the medial and mediolateral regions of the segment. The medial branch, which innervates the body wall and medial parts of the VIM (Fig. 1), has been designated the body nerve (BN) (*9*). Electrical activity of the receptor can be detected in the BN and in the main abdominal nerve in regions anterior to the junction with the BN.

Electrical activity in the BN was recorded by looping a portion of the nerve into an extracellular suction electrode, the diameter of which approximated the diameter of the nerve (*10*). With the aid of a micromanipulator, a glass probe was used to stretch or compress different parts of the abdomen. When this probe was placed on or near the junction of the BN and VIM, a sustained discharge of a single electrical unit occurred. This discharge continued as long as pressure was applied to this location and ceased immediately when pressure was removed by lifting the probe. Lightly touching the VIM resulted in a sustained low-frequency discharge. As pressure was increased by lowering the probe onto the VIM and the underlying body wall, the frequency of discharge increased (Fig. 2). This increase in electrical activity was not due to stretch of the VIM. Stretching the VIM without applying pressure to the body wall under the VIM produced, at best, irregularly occurring phasic discharges that could have resulted from inadvertent movement of the BN. Further, the region of sensitivity was relatively small. Application of pressure to areas greater than 50 to 100 μm from the most sensitive location failed to elicit electrical activity.

Methylene blue staining (*11*) revealed the

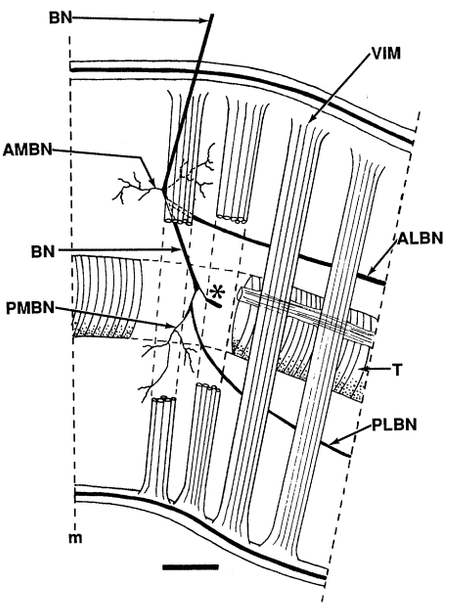

Fig. 1. Dorsal view of the ventral body wall and associated structures located to the right of the midline (m) in segment four illustrating the course of the body nerve (BN) delineated by methylene blue staining. Fat body and portions of the ventral intersegmental muscles (VIM) and segmental trachea (T) have been omitted for clarity. In each abdominal segment two to five, the BN forms an anterior medial (AMBN), an anterior lateral (ALBN), a posterior medial (PMBN), and a posterior lateral (PLBN) nerve branch. The elongated plaque-like structure (marked with an asterisk) is attached by a small branch to the BN near the location of the posterior branches. Scale bar, 200 μm.

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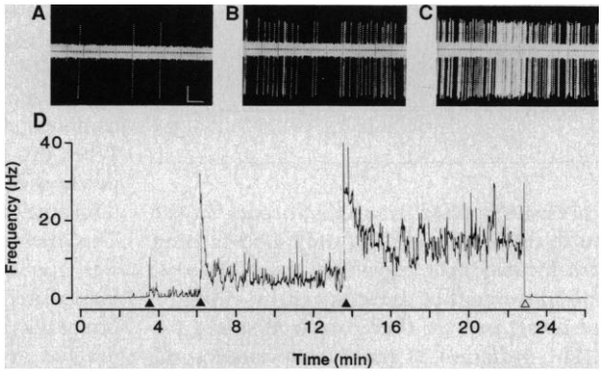

Fig. 2. Discharge rate of action potentials recorded extracellularly from an abdominal nerve of *Rhodnius* as pressure was applied internally with a blunt glass probe to the region of greatest sensitivity in segment four. Oscilloscope traces representing activity of the single electrical unit were taken at (**A**) 2 min, (**B**) 10 min, and (**C**) 18 min during the time delineated in (D). Vertical scale bar, 200 μV in (A), (B), and (C); horizontal scale bar, 2 s in (A) and (B) and 1 s in (C). (**D**) Changes in the discharge frequency measured by a rate interval analyzer as the number of spikes per second versus time. Pressure was applied (closed triangles) by gently lowering the probe onto the sensitive region, first at 3.5 min, then at approximately 6 and 14 min. At each of these times pressure was increased in a stepwise fashion and removed (open triangle) at 23 min.

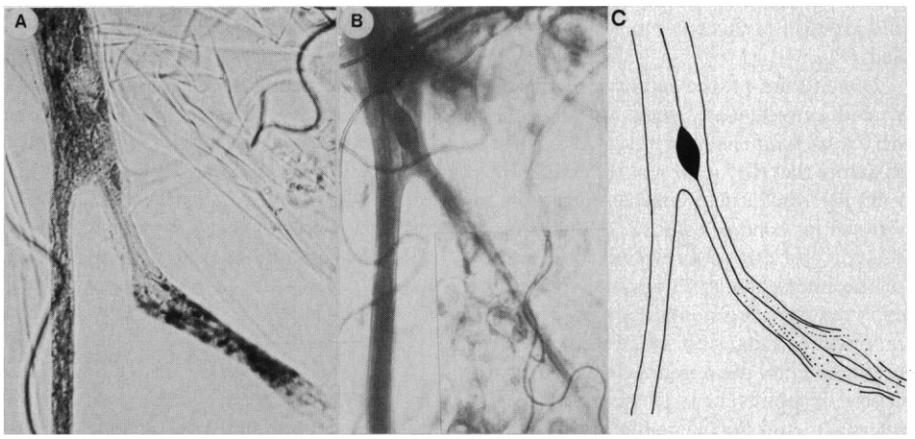

Fig. 3. The plaque-like structure embedded in the body wall (lower right in all panels) is connected to the abdominal nerve by a narrow strand. (**A**) Methylene blue–stained whole mount delineating the nerve processes and the full extent of the plaque embedded in the body wall. (**B**) An osmium-fixed preparation depicting a small strand terminating in a rodlike structure located within the plaque. The fine strand is continuous with an enlarged region indicative of a cell body in the BN. (**C**) Structure of the putative receptor as derived from several whole mounts stained with methylene blue or fixed with osmium.

BN to have six principal branches (Fig. 1). Upon reaching the level of its corresponding segment, the BN extended over the medial anterior end of the most medial bundle of the VIM sending two branches into the body wall at this location. One branch innervated the anterior medial region of the body wall, and the other, the lateral region. Posteriorly, the BN gave rise to another set of medial and lateral branches and sent a fifth branch dorsally to innervate the VIM (not shown in Fig. 1). The sixth branch of the BN (marked with an asterisk in Fig. 1) ended abruptly in the body wall as an elongated plaque-like structure (Fig. 3A). Although the precise location of this plaque-like structure varied between animals, it was always associated with the area of maximum sensitivity to applied pressure identifying the plaque as an integral part of the receptor.

Some details of the plaque-like structure were obtained from light microscopic observations of whole mounts of osmium-fixed preparations (*12*). Running longitudinally along the center of the plaque was a thin strand attached at its most distal end to a rodlike structure (Fig. 3B). The thin strand was continuous with an enlarged region, indicative of a cell body, located in the BN. Thus, the plaque-like structure embedded in the body wall may be associated with the dendritic processes of the sensory receptor, and the region along the BN, directly attached by a short branch to this structure, could encompass the cell body of the sensory receptor. The plaque's overall morphological appearance, as derived from methylene blue–stained and osmium-fixed preparations (Fig. 3C), suggests that this putative receptor is well designed to respond to pressure applied from above in much the same manner as the vertebrate Pacinian corpuscle. Moreover, the position of this structure is ideal for monitoring the distension of the stomach. Being situated to one side of the ventral midline in the central regions of segments two to five, it can detect pressure exerted on the body wall by the constant churning movements of the stomach. Conversely, corresponding regions in segments one and six in adults are not in a position to be greatly affected by movements of the stomach, and these segments appear to lack these plaque-like structures.

The identification of a receptor responding tonically to the internal pressure created by the distension of the stomach fills an important gap in the study of the control of hormone release in Hemiptera and other insects. Although some studies have provided evidence for receptors responding to stretch of the abdomen in *Rhodnius* (*6, 13*), these receptors appeared to adapt rapidly and could only be used to signal short-term events (*7*). The tonically discharging receptor described here is capable of continuously monitoring distension, and is well suited for controlling events occurring through a longer term, such as the release of PTTH or JH. It is readily apparent why such a structure has gone undetected. It responds to pressure rather than stretch of the intersegmental membranes, and it is hidden under the large segmental trachea and the VIM. It remains to be seen whether similar structures occur in other insects in which hormone release is dependent upon size expressed by abdominal distension (*5*).

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A Defective HSV-1 Vector Expresses *Escherichia coli* β-Galactosidase in Cultured Peripheral Neurons

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A defective herpes simplex virus 1 (HSV-1) vector, pHSVlac, has been developed that contains a transcription unit that places the *Escherichia coli lacZ* gene under the control of the HSV-1 immediate early 4/5 promoter. The vector pHSVlac was propagated with the HSV-1 temperature-sensitive mutant ts K as helper virus. Infection of neurons from rat superior cervical ganglia and dorsal root ganglia in primary culture resulted in stable expression of high levels of β-galactosidase without cell death. These HSV-1 vectors should be useful for introducing genes into postmitotic cells, such as neurons, in vitro and in vivo.

METHODS OF DELIVERING GENES into the cells of the nervous system are required before the functions of cloned neuronal genes may be studied. Of the four approaches used to introduce genes into cells [the frog oocyte microinjection system (*1*), transgenic mice (*2*), transfection of DNA directly into cells (*3*), and retrovirus vectors (*4*)], none can deliver a gene directly into nonmitotic cells. Here we report that pHSVlac, a defective HSV-1 vector that expresses the *Escherichia coli lacZ* gene from the HSV-1 immediate early (IE) 4/5 promoter, can infect peripheral neurons in primary culture and stably express high levels of β-galactosidase.

HSV-1 has great promise as a virus vector system (*5*). It has a wide host range; HSV-1 infects many cell types in mammals and birds (*6*). In addition, HSV-1 infects postmitotic neurons in adult animals and can be maintained indefinitely in a latent state (7). Latent HSV-1 is quiescent: HSV-1 gene expression is limited at most to the IE genes and a latency-associated transcript (*8*), DNA replication does not occur (7), and no progeny virus are produced (7). Electrophysiological properties are unaltered in latently infected neurons (*9*).

In virions, HSV-1 vectors are composed of head-to-tail repeats (*10*). The repeats are 5 to 15 kb in size, for up to 150 kb, which is the size of the HSV-1 genome. The vectors are maintained because of their growth advantage over the helper virus; HSV-1 contains three origins of DNA replication (ori), or one ori every 50 kb, whereas a vector contains one ori every 5 to 15 kb (*10*).

HSV-1 vectors have been propagated in a virus stock with wild-type HSV-1 as helper virus (*10*). The wild-type HSV-1 in the virus stock invariably causes cell death. However, intracerebral injection (*11*) and infection of mouse neuroblastoma cells (*12*) with HSV-1 temperature-sensitive (ts) mutants allow persistence of the virus without cell death. We obtained HSV-1 vectors that can infect cells without causing cell death by using ts mutants of HSV-1 as helper virus (*13*). At the restrictive temperature of 37° or 39°C the ts mutations block the lytic cycle and thereby prevent cell damage. Virus is grown at the permissive temperature of 31°C.

The 8.1-kb defective HSV-1 vector, pHSVlac, is shown in Fig. 1. The vector pHSVlac contains three kinds of genetic elements: (i) sequences that allow propagation of pHSVlac in *E. coli* (the ampicillin resistance gene and the *col* E1 *ori*); (ii) sequences from HSV-1 that support propagation of pHSVlac in an HSV-1 virus stock [the HSV-1 ori_s, an HSV-1 *ori* (*14*), and the HSV-1 "a" sequence, the packaging site (*15*)]; and (iii) a transcription unit. The components of the transcription unit are the HSV-1 IE 4/5 promoter (*14*), the intervening sequence following that promoter, the *E. coli lacZ* gene (*16*), and the SV40 early region polyadenylation site (*16*). The *E. coli lacZ* gene encodes a β-galactosidase absent from mammalian cells, thereby providing an assay for expression of the transcription unit in pHSVlac (*17*). pHSVlac DNA was packaged into HSV-1 virus particles, with HSV-1 strain 17 ts K (*18*) as helper virus. ts K has a mutation in the IE 3 gene, has an immediate early phenotype, and is not permissive for DNA replication.

To determine if pHSVlac virus stock could infect neurons and express β-galactosidase, we prepared primary cultures of dissociated neurons (*19*) from dorsal root ganglia and superior cervical ganglia of newborn rats. Cultures were infected with pHSVlac virus stock, then incubated for 24 hours at 37°C, fixed, and assayed for β-galactosidase activity in situ (*17*) by using the chromogenic substrate 5-bromo-4-chloro-3-indolyl β-D-galactoside (X-gal). At a multiplicity of infection (MOI) of 0.1 to 0.4 of pHSVlac about 38% of the cells in the cultures of dorsal root ganglia (Fig. 2, A to C) and 11% of the cells in the cultures of superior cervical ganglia (Fig. 2, D to F) were β-galactosidase–positive. Experiments performed at higher ratios of pHSVlac virus to cells (MOI 2) resulted in expression of β-galactosidase in virtually every cell. Cultures infected with ts K alone or mock-infected cultures contained less than 0.2% β-galactosidase–positive cells.

Most of the β-galactosidase–positive cells shown in Fig. 2 have the morphological characteristics of neurons. However, β-galactosidase–positive cells that resembled glia were also observed. To determine whether some of the β-galactosidase–positive cells were indeed neurons, we performed an ex-

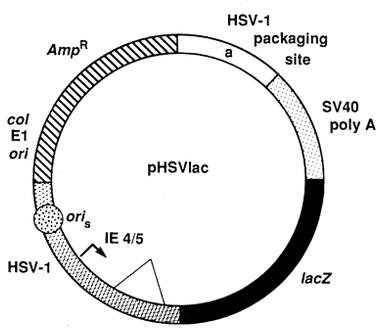

Fig. 1. The structure of pHSVlac. The clear region contains the HSV-1 a segment, nucleotides 127 to 1132, the packaging site (*15*). The crosshatched region symbolizes the HSV-1 c region, nucleotides 47 to 1066 (*14*). pHSVlac was constructed (*13*) from pCH110 (*16*).

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