ZOOLOGICAL SCIENCE 18: 465–473 (2001)

Amino Acid-induced Reflexes and Their Neural Pathways in an Opisthobranch Mollusc *Pleurobranchaea japonica*

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ABSTRACT—Certain behavioral acts of *Pleurobranchaea japonica* may be elicited in response to chemical stimulants applied to the rhinophores, tentacles or oral veil. These include feeding and escape. Upon treatment with chemical stimulants, the organs respond directly by extending or withdrawing. Beyond this, aversive responses involved withdrawal of the rhinophores, tentacles and oral veil plus contraction of the whole body. Feeding behavior was elicited by glycine, phenylalanine, proline, aspartic acid, alanine, asparagine, tryptophan and glutamine. An aversive response was induced by glutamate. Neural pathways responsible for these chemoreceptive reflexes have been identified. In addition to known nerves, a newly identified pair of nerves, the second pedal nerves arise from the pedal ganglion and send branches to the three organs. The nerves contain afferent pathways for chemoreception of the organs and efferent motor pathways for the movements of the organs. The rhinophore ganglion is responsible for the glutamate-induced contraction of the rhinophore. This withdrawal reflex of the rhinophore is under inhibitory control, exerted by the cerebral ganglion through the rhinophore nerve, and under an excitatory influence, emanating from the pedal ganglion through the second pedal nerve.

INTRODUCTION

Chemical receptors play an important role in feeding, escape, homing and mating behavior in opisthobranch molluscs (review by Croll, 1983). A variety of responses for feeding behavior were mediated by receptors in the paired rhinophores (posterior tentacles), the paired tentacles (anterior tentacles) and an oral veil; all of which are located at the anterior region of the body, in a marine carnivorous opisthobranch mollusc *Pleurobranchaea californica* (Davis and Mpitsos, 1971; Lee *et al.*, 1974; Davis and Matera, 1982; see also the review by Croll, 1983).

When chemical stimuli were applied to the chemoreceptive organs in *P. japonica*, the organs showed extensional and withdrawal movements themselves. When squid extracts were ejected in front of the animals, the animals displayed extension of the rhinophores and tentacles, and waving of the oral veil, followed by extension of the proboscis.

In *P. californica*, three pairs of nerves that originating from the cerebropleural ganglion have been shown to be afferent pathways from the three chemoreceptive organs (Lee and Liegeois, 1974). Bicker *et al.* (1982) recorded impulses from the afferent nerves in response to glycine and alanine applied to the organs.

In the present study, we have examined responses to a variety of amino acids in *P. japonica*. The seven amino acids that induced feeding behavior in *P. californica* were effective to *P. japonica*. Glutamate was an amino acid ineffective for feeding responses (Bicker *et al.* 1982), but, in *P. japonica*, induced clear aversive responses, such as withdrawals of the rhinophores, tentacles and oral veil, and contraction of the whole body. We have searched for neural pathways responsible for the chemoreceptive reflexes of three organs in *P. japonica*. We have newly identified a pair of the second pedal nerves arising from both right and left pedal ganglia which were involved in the pathways, besides cerebral nerves previously identified as chemoreceptor nerves in *P. californica* (Lee and Liegeois, 1974; Bicker *et al.*, 1982). The nerves may serve as afferent and efferent pathways for chemoreception-induced reflexive movements.

MATERIALS AND METHODS

Pleurobranchaea japonica weighing 30–80 g were collected from fishermen's traps at the bottom of Tokyo Bay or Sagami Bay. They were kept in natural sea water (SW) tanks of a laboratory aquarium at about 13°C under light and dark cycles of 12 : 12. The bottoms of the SW tanks were covered with a sand layer about 10 cm thick. Animals were fed with sliced pieces of squid and shrimp. Behavioral and electrophysiological experiments were performed at about 13°C.

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Solutions containing squid extracts and amino acids

Solutions containing squid extracts (SE) were made by immersing one gram squid body slice in approximately 100 ml of SW for 30 minutes and filtering the SW.

Amino acids used were alanine, asparagine, asparatate, glutamate, glutamine, glycine, phenylalanine, proline, taurine, tryptophan and valine (Wako Pure Chemical Industries, Ltd.). Amino acids were dissolved in SW at 10^{-1} M to 10^{-5} M for chemical stimulants. Some amino acid solutions (10^{-2} M) were prepared by diluting a stock solution (10^{-1} M in DW) with SW.

Behavioral experiments

Animals were starved for two days before experiments. Experiments were performed during a light phase (9 am to 9 pm). Thirty minutes before each experiment the animals were put in a 1 liter experimental chamber filled with fresh SW.

Stimulant solutions (1 ml in volume) were applied to the anterior end of each animal through a glass pipette, whose tip was placed at 1.5 cm apart from the animal. Solutions were applied carefully to minimize mechanical influence on the animal.

The behavioral responses of animals were observed for three minutes after application of solutions. Chemoreceptive responses were first detected as orientation of the rhinophores toward stimulants and then waving of the oral veil. Feeding behavior was characterized according to three behavioral components which occurred successively: (I) orientation of the oral veil to the food stimulus, (II) extension of the proboscis and (III) biting movements of the odontophores (cf. Davis and Mpitsos, 1971; Bicker *et al.*, 1982). Aversive behavior was characterized by withdrawal of the rhinophores, tentacles, oral veil and gill, or contraction of the whole body. Aversive responses were observed visually for intact animals or detected by recording mechanograms of isolated organs.

After behavioral experiments, the animals were fed with a piece of squid or shrimp. When they did not eat, the data obtained from them were discarded.

Anatomy

Before dissection, animals were anesthetized by injecting into the hemocoel with an ice-cold isotonic $MgCl_2$ solution (0.36 mol / l) of about 30% of the body weight. An animal was pinned to the soft plastic bottom of a dish filled with ice-cold SW which contained three times as much Mg^{++}ions as the concentration in natural SW. The left side of the dorsal body wall was carefully incised, and the digestive organs including the acid gland were removed. The preparation was stained with methylene blue and fixed in a 4% ammonium molybdate solution in SW overnight. After washing out the fixative in running tap water, the preparations were repeatedly stained with the dye during dissection in the tap water (cf. Matsumura and Kuwasawa, 1996).

Preparations for electrophysiological experiments

Two types of preparations were used. One was an isolated chemoreceptor organ, which lacked a connection to the central nervous system (CNS). For the isolation of the rhinophore, tentacle, or oral veil, the cerebral nerves innervating the organs were cut near the origin of the nerves from the CNS, as was the second pedal nerve (P2). The other was a preparation of a rhinophore isolated together

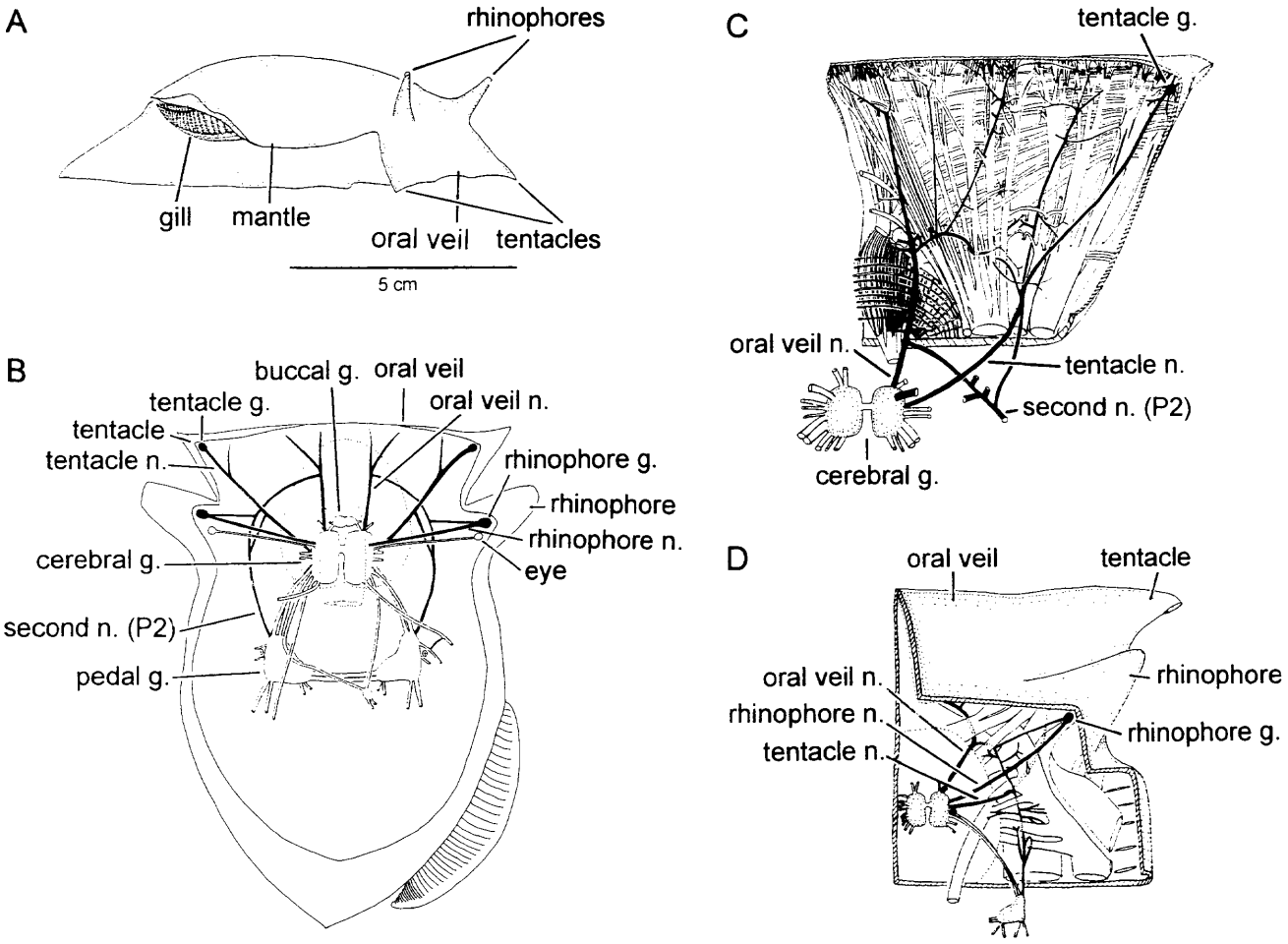

Fig. 1. Schematic drawings of the structure and innervation of chemoreceptor organs in *Pleurobranchaea japonica*. (A) The anterior portion of the body has three chemoreceptor organs, the rhinophores, tentacles and oral veil. (B) The rhinophore, tentacle and oral veil nerves arise from the cerebral ganglion. The second pedal nerve (P2) joins to the three nerves through its branches. (C) Anatomy of the periphery of the oral veil and tentacle nerves. (D) Anatomy of the periphery of the rhinophore nerve and P2. g., ganglion; n.,nerve.

with the CNS. The preparation was removed and pinned to the soft plastic bottom of an experimental chamber (5 ml in capacity). The specimen was perfused constantly with filtered normal SW during an experiment.

Amino acid solutions and SE were applied by switching a three-way valve on a perfusion line. The rhinophore and tentacle ganglia are located at the base of their own organs (Fig. 1). In order to prevent a direct effect of the stimulants on the ganglia, the stimulants were applied to the top of a rhinophore or tentacle exposed in a chamber through a hole in a thin rubber sheet. When a preparation of the rhinophore with the CNS was used, the rhinophore and CNS were placed in different compartments separated by a thin plastic partition. The rhinophore nerve and P2 were passed through U shaped-slits in the partition, insulated with vaselin. To block chemical transmission in the CNS, the CNS was bathed in high Mg^{++} (150 mM) SW.

Extracellular recording of afferent impulses

A glass capillary suction electrode equipped with an Ag-AgCl wire was used for recording extracellular impulses from nerves. The rhinophore, tentacle, oral veil nerves and P2 were cut, and the distal cut-stumps were introduced into the suction electrodes. Electrical signals were fed into an AC-amplifier and recorded on a memory oscilloscope, a chart recorder, and a digital data recorder.

Recording of mechanograms

Mechanograms were recorded with a strain gauge mechanotransducer. Fine silk threads ran from the distal end of each organ to the transducer, while the proximal end of each organ was pinned to the soft plastic bottom of the experimental chamber. The signal from the mechanotransducer was applied through a DC-amplifier to a pen-recorder.

Electrical stimulation

A glass capillary suction electrode was used to apply stimulus pulses to nerves. The electrode was supplied pulses from a stimulator through an isolator.

RESULTS

Responses to amino acids

Application of SE to the rhinophores, tentacles or oral veil of *P. japonica* induced chemoreceptive responses followed by feeding responses, such as (I) orientation of the oral veil to the food stimulus, (II) extension of the proboscis and (III) biting movements of the odontophores (cf. Davis and Mpitsos, 1971; Bicker *et al.*, 1982).

We tested eleven kinds of amino acids, alanine, asparagine, asparatate, glutamate, glutamine, glycine, phenylalanine, proline, taurine, tryptophan and valine. In response to all the amino acids, either feeding or aversive reflexes induced by solutions of high concentrations were stronger than those by solutions of low concentrations.

Table 1 summarizes the numbers of animals, which showed feeding, aversive or no responses to the applied amino acids (10^{-4} M to 10^{-1} M). The numbers of animals which showed feeding responses were reported for animals which responded to amino acids with at least one of the specified successive feeding behavioral components (I–III). Alanine, asparagine, asparatate, glutamine, glycine, phenylalanine, proline and tryptophan induced feeding behavior in more than half of the animals, and glycine induced the responses most frequently. Glutamate, valine and taurine induced aversive responses. Glutamate evoked the most frequent and clearest aversive responses (see Table 1, see also Fig. 8), which were perceived as withdrawal responses of the rhinophores, tentacles, oral veil, gill, mantle, foot and body wall. Even 10^{-4} M glutamate induced aversive responses, though 10^{-5} M induced chemoreceptive responses only on the rhinophores and oral veil. The rhinophores showed the clearest withdrawal responses to glutamate among the organs and regions. Valine and taurine induced aversive responses in six of ten animals tested.

Neuroanatomy

We carried out neuroanatomical studies to reveal the innervation of the rhinophores, tentacles and oral veil in detail. Schematic drawings of the CNS and innervation of the chemosensory organs are illustrated in Fig. 1. The rhinophore nerve originates from the dorsal side of the cerebral ganglion, and runs to the rhinophore ganglion that is located at the base of the rhinophore. The tentacle nerve extends from the cere-

Table 1. Behavioral responses of intact animals to amino acids

Amino acid	Total specimens	Feeding	Aversive	No or indistinct responses
Gly	31	27	0	4
Phe	24	17	0	7
*Pro	30	21	0	9
Asp	10	7	0	3
Ala	37	24	0	13
Asn	25	17	0	8
Trp	10	6	0	4
Gln	27	16	0	11
**Glu	10	0	10	0
Val	10	0	6	4
***Tau	10	0	6	4

Numerals, number of animals
*, no response for 80% of animals in *P. californica* (Bicker *et al.* 1982)
**, no response for 100% of animals in *P. californica* (Bicker *et al.* 1982)
***, aversive responses in *P. californica* (Gillette *et al.* 1991)
Feeding, feeding responses; Aversive, aversive responses.

bral ganglion and one of the branches runs to the tentacle ganglion located at the base of the tentacle. The oral veil nerve is identical to the large oral veil nerve in *P. californica* (Davis *et al.*, 1973). That nerve extends from an anterior lateral site on the cerebral ganglion, and runs to the anterior margin of the oral veil, sending many fine branches to dorsal and ventral regions of the oral veil. The gloss anatomy of the peripheral nerves arising from the cerebral ganglion (which was referred to as the cerebropleural ganglion in *P. californica*) is almost the same as that in *P. californica* (Davis *et al.*, 1973; Lee *et al.*, 1974).

The P2 extends from the pedal ganglion at a site near the cerebro-pedal connectives and has many branches including ones joining the oral veil and tentacle nerves, and the rhinophore ganglion. Thus, the P2 sends its branches to all the three chemoreceptor organs.

Afferent impulses from the chemoreceptor organs

To identify chemosensory pathways, we recorded impulses of the rhinophore, tentacle and oral veil nerves and P2. An example of impulses recorded from a distal stump of the rhinophore nerve is shown in Fig. 2A. To examine the effects of alternation in flow rate of perfusates on the rhinophore, switching the valve was simulated with plain SW. Application of plain SW did not change firing activity for several kinds of impulses (upper trace), while application of SE to the rhinophore increased impulse activity in the rhinophore

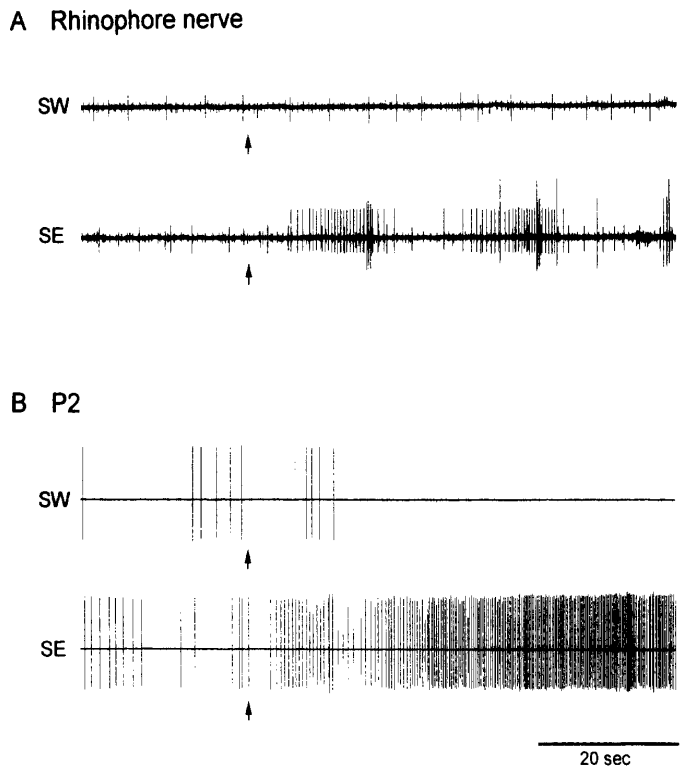

Fig. 2. Afferent impulses recorded from a rhinophore nerve and P2. (A) SW applied to the rhinophore induced no change in impulse discharges of the rhinophore nerve (upper trace), while SE increased impulse discharges (lower trace). (B) SE increased impulse activity of the P2 (lower trace), but SW did not (upper trace). Arrows, the beginning of SW or SE application.

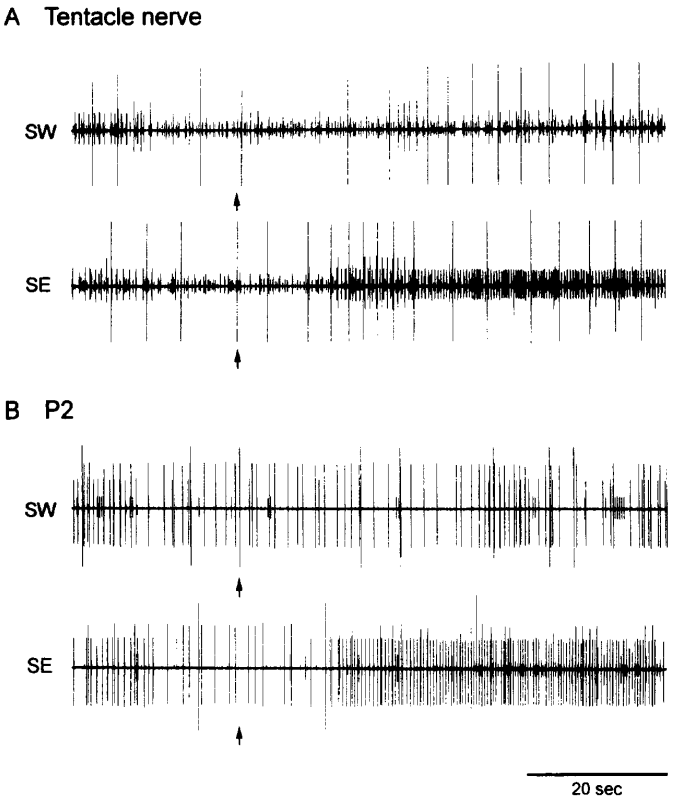

Fig. 3. Afferent impulses recorded from a tentacle nerve and P2. (A) SE applied to the tentacle increased impulse discharges of the tentacle nerve (lower trace), but SW alone did not (upper trace). (B) Application of SE to the tentacle increased impulse activity of the P2 (lower trace).

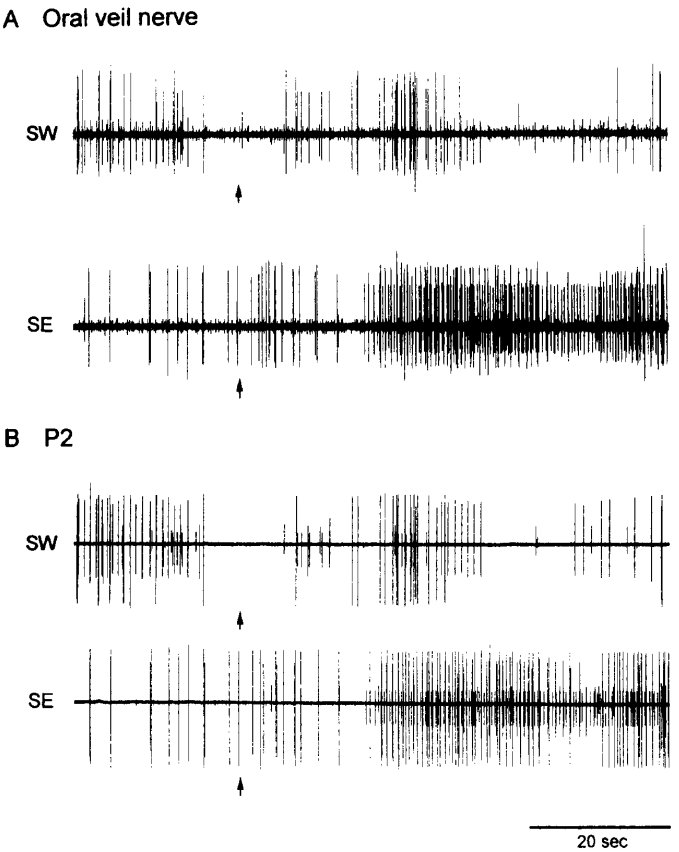

Fig. 4. Afferent impulses recorded from an oral veil nerve and P2. (A) SE applied to the oral veil increased impulse activity of the oral veil nerve (lower trace), but SW alone did not (upper trace). (B) SE increased impulse activity of the P2 (lower trace).

nerve (lower trace). Figure 2B shows impulses recorded from a distal stump of the P2. SE applied to the rhinophore increased impulse discharges in the P2 (lower trace). The results show that both the rhinophore nerve and P2 contain afferent chemosensory axons from the rhinophore.

Fig. 3 shows impulses recorded from distal stumps of the tentacle nerve (A) and P2 (B). SE applied to the tentacle increased impulse activity of both the tentacle nerve and P2 (each of the lower traces). The results show that both the tentacle nerve and P2 contain afferent chemosensory axons from the tentacle.

Impulses recorded from distal stumps of the oral veil nerve and P2 are shown in Fig. 4. Application of SE to the oral veil increased firing activity of both the nerve (each of the lower traces). The results show that both the oral veil nerve and P2 contain afferent chemosensory axons from the oral veil.

Thus, SE induced impulses in all the nerves from the rhinophore, tentacle and oral veil, indicating that each organ sends chemosensory signals to both the cerebral and pedal ganglia.

Motor nerves innervating the chemoreceptor organs

Fig. 5 shows typical mechanograms recorded from the rhinophore in response to electrical stimulation of a distal stump of the rhinophore nerve (A) or P2 (B). Stimulation of rhinophore nerve elicited contraction of the rhinophore, which had dual phases with a sharp first peak followed by a slow second peak (A1). Stimuli at a low intensity elicited relaxation of the rhinophore (A2). Stimulation of P2 with strong intensity elicited contraction of the rhinophore, while stimulation of the nerve with a low intensity relaxed the rhinophore (B2). These results indicate that both the nerves contain excitatory and inhibitory axons acting on the rhinophore.

Fig. 6 shows mechanograms of the tentacle. Stimuli applied to a distal stump of the tentacle nerve elicited con-

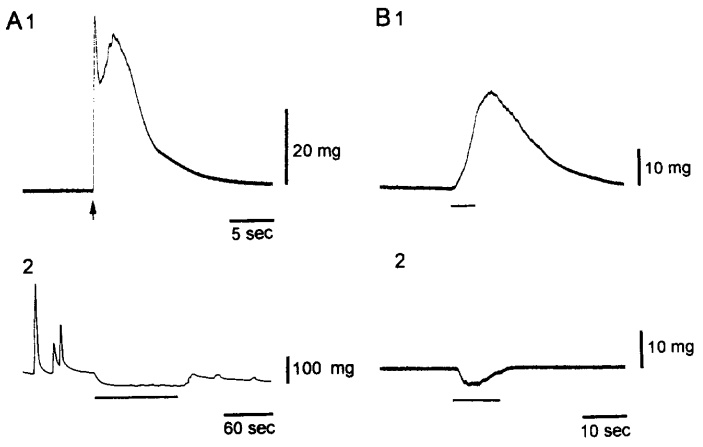

Fig. 5. Mechanograms recorded from a rhinophore. (A) A single electrical stimulus (7V, 400 μsec) to the rhinophore nerve induced a contraction of the organ (1), while repetitive stimulation of the nerve at 1Hz (2V, 200 μsec) induced a relaxation (2). (B) Repetitive stimulation of the P2 at 5Hz (6V, 500 μsec) induced a contraction of the rhinophore (1), while stimulation at 20Hz (4V, 200 μsec) caused a relaxation (2). Arrow, timing of a single stimulus. Horizontal bars, repetitive stimulation.

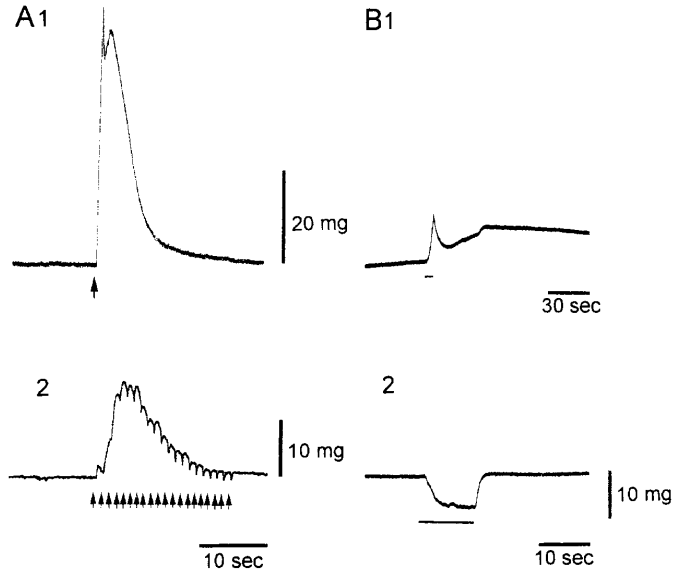

Fig. 6. Mechanograms recorded from a tentacle. (A) A single stimulus (7V, 1msec) applied to the tentacle nerve evoked a large contraction of the tentacle (1), while the contraction was reduced by a train of relaxations during a period of repetitive stimuli (9.5V, 130 μsec, 1Hz) (2). (B) Repetitive stimulation of the P2 at 5Hz (6V, 500 μsec) evoked a contraction of the tentacle (1), while stimulation of 20Hz (6V, 300 μsec) caused a relaxation (2). Arrows, timing of stimuli. Horizontal bars, repetitive stimulation.

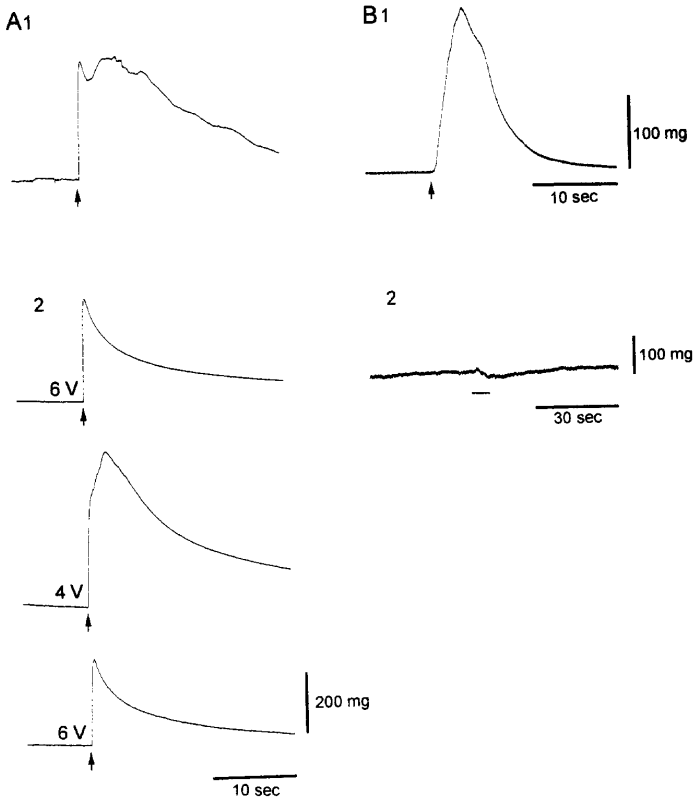

Fig. 7. Mechanograms recorded from an oral veil. (A) Contractions evoked by electrical stimuli applied to the oral veil nerve. Traces (2) show part of records when electrical stimuli were applied at intervals of 3 minutes. Stimuli at intensity of 6V and 4V were presented alternately for four times respectively. Note that contraction evoked by a weak stimulus was larger and longer (middle trace) than that evoked by a strong stimulus (upper and bottom traces). (B) A single stimulus (8V, 1msec) applied to the P2 evoked contraction (1), while repetitive stimulation (8V, 200 μsec, 1Hz) evoked relaxation (2).

traction of the tentacle with a sharp first peak and slow second peak (A1). When a train of stimulation was applied the tentacle contracted in an early period, and relaxed in the late period (A2). Stimulation of the P2 also elicited both contraction (B1) and relaxation (B2) of the tentacle. These results may show that the nerves include both excitatory and inhibitory axons for the tentacle.

Fig. 7 shows mechanograms of the oral veil. Stimuli applied to a distal stump of the oral veil nerve elicited contraction of dual phases with a sharp first peak and a slow second peak (A1). A2 shows contractions evoked by alternate stimulation with 6V and 4V, at 3 min intervals. Contractions evoked by 4V stimulation were larger than those evoked by 6V. Means and SD of peak amplitudes of contractions evoked by 4V and 6V stimulation were 527.8±61.7 mg (n=4), 343.3±24.2 mg (n=4), respectively. The contractions might be suppressed by inhibitory axons excited by stimuli of higher intensity (6V). The oral veil nerve may include both excitatory and inhibitory axons. Strong stimulation of P2 evoked contraction of the oral veil (B1), while a weak stimulation relaxed the organ (B2). These results may indicate that the P2 sends both excitatory and inhibitory axons to the oral veil.

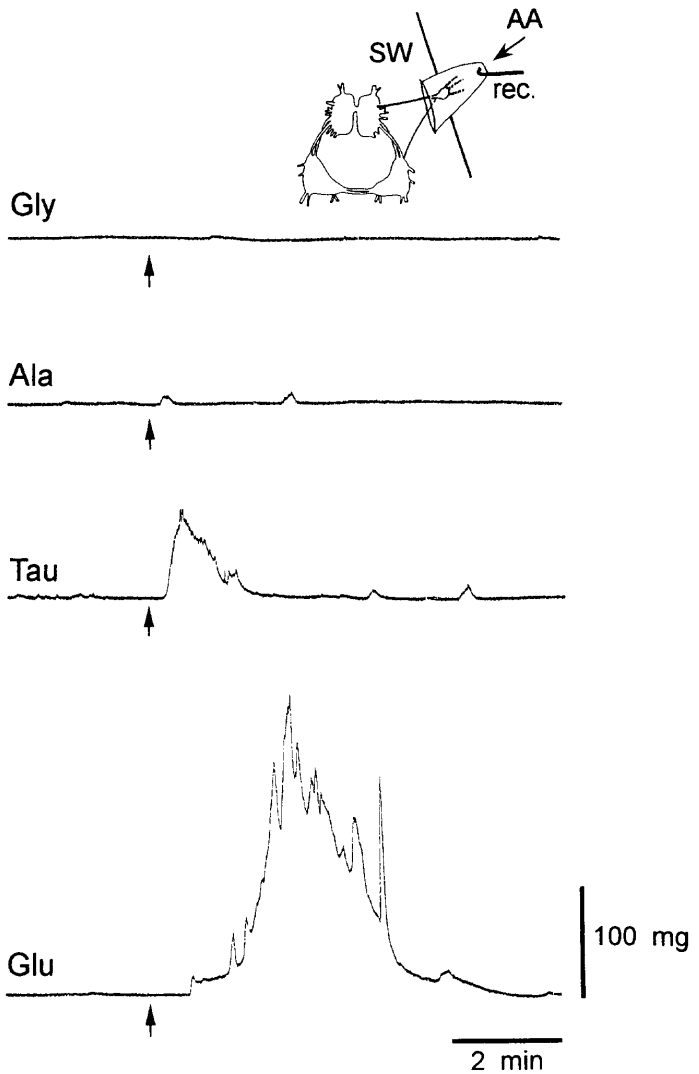

Fig. 8. Mechanograms showing contraction of the rhinophore induced by amino acids (AA, 10^{-2} M). A much larger contraction of the rhinophore was evoked by glutamate (Glu) than by the other three amino acids, glycine (Gly), alanine (Ala) and taurine (Tau). Arrows indicate the beginning of amino acid perfusion of a period of 60 sec. Inset shows preparation used for experiments.

Neural mechanisms for withdrawal responses of the rhinophore

Fig. 8 shows mechanograms of the rhinophore with the CNS. Glycine and alanine failed to contract the rhinophore. Taurine induced considerable contraction of the rhinophore. Glutamate evoked much larger contraction of the rhinophore (bottom trace).

We examined local withdrawal reflexes in isolated preparations of the rhinophore retaining the rhinophore ganglion but lacking the CNS. Even in the isolated rhinophore, glutamate evoked contraction of the rhinophore (Fig. 9A, upper trace). After removal of the rhinophore ganglion from the preparation glutamate evoked little contraction (lower trace). Thus, the major part of the large reflex may be attributed to the rhinophore ganglion.

We then examined the contribution of the CNS to the reflex using preparations retaining the cerebral, pedal and rhinophore ganglia. When the CNS was bathed in high Mg^{++} SW, the amplitude of contraction induced by glutamate became larger than that when the CNS was bathed in the normal SW (Fig. 9B). This suggests that the CNS contains

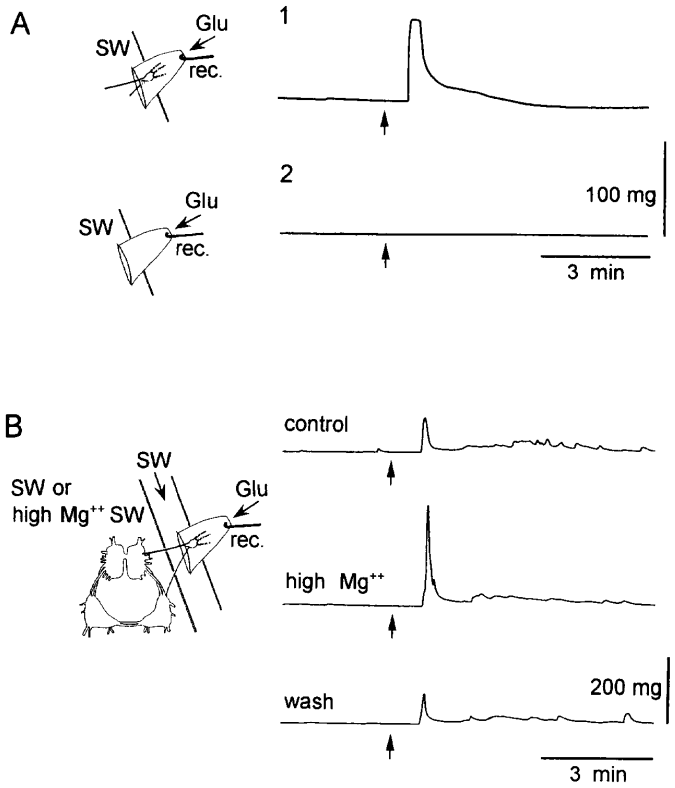

Fig. 9. (A) Effects of removal of ganglia on contraction of the rhinophore. Even after both the cerebral and pedal ganglia were removed, 10^{-2}M glutamate application to the rhinophore induced contraction of the organ (upper trace). After removal of the rhinophore ganglion, glutamate evoked little contraction (lower trace). (B) In the rhinophore with CNS, bathing the cerebral and pedal ganglia in high Mg^{++} SW augmented the contraction induced by glutamate (arrow in middle trace).

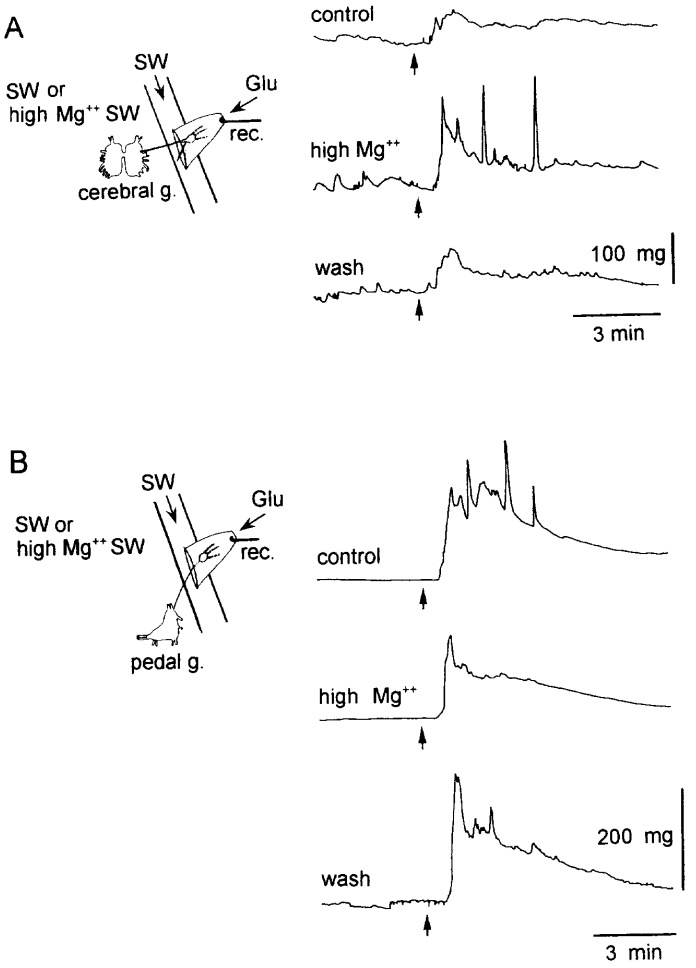

Fig. 10. Effects of bathing the cerebral and pedal ganglia in high Mg^{++} SW. (A) Bathing the cerebral ganglion in high Mg^{++} SW augmented the contraction induced by 10^{-2}M glutamate stimulation (middle trace). (B) Bathing the pedal ganglion in high Mg^{++} SW decrease the amplitude and frequency of the contraction induced by 10^{-2}M glutamate (arrow in middle trace).

suppressive mechanisms for the withdrawal reflex of the rhinophore induced by glutamate.

We examined whether the cerebral or pedal ganglion is responsible for the suppression of withdrawal responses. In the rhinophore preparation with the cerebral ganglion and lacking the pedal ganglion; when the cerebral ganglion were bathed in high Mg^{++} SW, the amplitude of glutamate-induced contraction became larger than the control (Fig. 10A). In the rhinophore with the pedal ganglion and lacking the cerebral ganglion, when the pedal ganglion was bathed in high Mg^{++} SW, the amplitude of glutamate contraction became smaller than the control (Fig. 10B). These results may indicate that the pedal and cerebral ganglia were responsible for augmentation and suppression of withdrawal responses by the rhinophore ganglion, respectively.

DISCUSSION

Amino acids and behavioral responses

Bicker *et al.* (1982) showed that ten kinds of amino acids elicited reliable feeding responses in *P. californica*, and that the strongest and most frequent responses were induced by seven amino acids (alanine, asparagine, asparatate, glutamine, glycine, phenylalanine and tryptophane). *P. japonica* was sensitive to all the eleven amino acids. We observed that the seven amino acids, which is most effective to *P. californica*, induced feeding behavior in more than half of the animals (Table 1). In *P. californica* proline (10^{-1} M) induced feeding responses only in two of ten animals (Bicker *et al.*, 1982), while in *P. japonica*, proline (even at 10^{-2}M) elicited feeding responses in twenty one of thirty animals (Table 1). Proline may be a member of amino acids responsible for induction of feeding behavior. In the present study, animals were starved for two days before experiments. Since Bicker *et al.* (1982) did not mention whether animals were starved or not, it is not clear whether this difference is due to different specimen or physiological conditions.

Glutamate, valine and taurine induced aversive responses (Table 1). Glutamate (10^{-4} to 10^{-1} M) evoked clear aversive responses involving withdrawal responses of the rhinophores (Fig. 8), tentacles, oral veil, gill, foot and body wall in *P. japonica*. Glutamate induced the clearest aversive responses in *P. japonica*. Gillette *et al.* (1991) reported that, in *P. californica*, taurine induced aversive responses. Valine also induced aversive responses in *P. japonica*, and the threshold concentration of valine (5×10^{-4} M) appeared to be lower than that of taurine.

Afferent and efferent neural pathways for chemoreceptor organs

The rhinophores, tentacles and oral veil are the most important organs required for chemoreception to trigger feeding behavior in *P. californica* (Davis and Mpitsos, 1971; Davis *et al.*, 1973; Lee *et al.*, 1974). Ciliated cells in the inner surface layer of these organs seem to have chemosensory ability (Davis and Matera, 1982; Matera and Davis, 1982). The rhinophore, tentacle and oral veil nerves, appear to be chemosensory nerves from the organs (Lee and Liegeois, 1974; Bicker *et al.*, 1982). In the present study, we found that another pair of nerves extending from the pedal ganglion sent branches to all three of the organs (Fig. 1B). Lee and Liegeois (1974) designated this nerve as the P2, and observed its branches innervating the tentacle and oral veil, though they did not identify any branch of the P2 extending to the rhinophore. Although they reported that the P2 did not respond to SE applied to the anterior region of the animal, the present study revealed that impulse discharges of the P2 were clearly increased by SE applied to the these organs (Fig. 2, 3, 4). The discharges lasted until SE was washed out. We may conclude that not only the nerves from the cerebral ganglion, but also the P2 contains chemosensory axons from all three chemoreceptor organs.

SE applied to the rhinophore (Fig. 2), tentacle (Fig. 3) and oral veil (Fig. 4) also activated the cerebral nerves from those organs. Bicker *et al.* (1982) and Lee and Liegeois (1974) reported identical results in *P. californica*. We may conclude that chemoreceptive signals from each of the three organs are sent to both the cerebral and pedal ganglia.

In *Aplysia,* the rhinophores, tentacles, and oral veil are chemosensory organs (Preston and Lee, 1973; Jahan-Parwar, 1972, 1975). All of the chemosensory nerves of these organs enter the cerebral ganglion (Jahan-Parwar, 1972; Audesirk and Audesirk, 1977; Chase, 1979). It remains to be determined whether or not *Aplysia* has a nerve equivalent to the P2 of the pedal ganglion.

Excitatory motor pathways to the chemoreceptor organs are as follows. (1) The rhinophore, tentacle and oral veil nerves are motor pathway for the rhinophore, tentacle and oral veil, respectively (Fig. 5A, 6A, 7A). (2) The P2 has motor pathways for all three organs (Fig. 5B, 6B, 7B). This means that each chemoreceptor organ receives polyneural excitatory motor innervation from the two central ganglia, the cerebral and pedal ganglia. The rhinophore, tentacle and oral veil also receive inhibitory motor innervation from both the cerebral and pedal ganglia (Fig. 5, 6, 7).

Neural mechanisms for chemoreception-induced withdrawal reflexes in the rhinophore

Application of glutamate to the anterior end of the body evoked aversive behavioral responses which included the withdrawal of the rhinophores. The rhinophore separated from the CNS contracted in response to application of glutamate (Fig. 9A). In this chemoreceptive reflex of the rhinophore, the rhinophore ganglion played a major part of the local reflex because only a faint contraction was observed after removal of the ganglion (Fig. 9A, lower trace). In *Aplysia*, the branchial ganglion in the gill includes motor neurons responsible for the pinnule withdrawal reflex (Kurokawa and Kuwasawa, 1985 a, b), and the siphon includes peripheral motor neurons inducing its withdrawal reflex (Bailey *et al.*, 1979). The rhinophore ganglion may include motor neurons mediating the withdrawal movements.

Glutamate evoked contraction of the rhinophore. Bathing the CNS in high Mg^{++} SW resulted in augmentation of the glutamate-induced contraction of the rhinophore (Fig. 9B), indicating that the CNS exerted suppressive effects on the withdrawal reflex of the rhinophore. Fredman and Jahan-Parwar (1977) reported, in *Aplysia*, that contraction of the tentacle induced by central motor neurons was augmented in high Mg^{++} SW. The present study shows that both the rhinophore nerve and P2 may contain both excitatory and inhibitory axons for the rhinophore contraction (Fig. 5). There may be functional differences between modulatory actions of the cerebral and pedal ganglia for contraction of the rhinophore. Withdrawal responses of the rhinophore induced by glutamate were mediated by the rhinophore ganglion. The rhinophore responses were under inhibitory control exerted by the cerebral ganglion through the rhinophore nerve, and excitatory control from the pedal ganglion through the P2 (Fig. 10). A possible mechanism is that high Mg^{++} SW might block an excitatory synaptic transmission to the inhibitory neurons in the CNS that suppress rhinophore muscle contraction or motor neurons of the rhinophore ganglion.

The tentacle and oral veil also received both excitatory and inhibitory innervation from both the cerebral and pedal ganglia (Fig. 6, 7). The isolated tentacle with the tentacle ganglion contracted in response to glutamate. In the tentacle with the CNS, bathing the CNS in the high Mg^{++} SW resulted in augmented its contraction in response to glutamate (unpublished data), thereby indicating that the CNS exerted suppressive effects on the withdrawal reflex of the tentacle, as in the rhinophore contraction. However, it remains to be determined whether the cerebral or pedal ganglia exerts inhibitory or excitatory influences on the withdrawal reflex of the tentacle. Unlike the rhinophore and tentacle, the oral veil has no peripheral ganglion. The oral veil with a connection to the CNS, contracted in response to glutamate. The results indicate that glutamate receptors in all the three chemoreceptor organs mediate aversive responses, although removal of the CNS from the organs greatly reduced contraction of the oral veil unlike the rhinophore and tentacle (unpublished data). Contributions of the cerebral and pedal ganglia to the withdrawal reflexes of the oral veil remain to be examined.

ACKNOWLEDGEMENTS

We express thanks to Prof. R. B. Hill for revising this manuscript. We also thank Messrs. T. Kayama and K. Shishikura for collecting animals. Contribution from the Shimoda Marine Research Center, No. 658.

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(Received January 9, 2001 / Accepted Feburary 2, 2001)