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Pharmacology, Biochemistry and Behavior 82 (2005) 678 – 685
PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR
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Effect of metabotropic glutamate receptor agonists and signal transduction modulators on feeding by a caterpillar

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Received 21 July 2005; received in revised form 7 November 2005; accepted 14 November 2005
Available online 20 December 2005

Abstract

Feeding in codling moth caterpillars was induced by the general glutamate receptor activator monosodium glutamate (MSG) and by three different mGluR agonists known to specifically stimulate different classes of vertebrate metabotropic glutamate receptors, including: (1S,3R)-ACPD, which stimulates group I mGluRs (2R,4R)-APDC, which stimulates group II mGluRs and L-AP4, which stimulates some group III mGluRs. Experiments exposing larvae to combinations of specific mGluR agonists and specific signal transduction modulators suggest that each tested mGluR uses a different signaling pathway. First, feeding stimulatory effects of (1S,3R)-ACPD were abolished by phospholipase C inhibitor, U 73122, but remained unaffected by adenylate cyclase activator, NKH 477, or phosphodiesterase inhibitor, Rolipram. Second, (2R,4R)-APDC induced feeding in presence of U 73122 or Rolipram, but lost its feeding stimulatory effects in presence of NKH 477. Finally, L-AP4 did not induce feeding in presence of Rolipram, but maintained its feeding stimulatory effects in presence of U 73122 or NKH 477. The activity of the general glutamate receptor activator MSG was abolished by NKH 477, and Rolipram. U 73122 did not affect MSG-stimulated feeding. These results suggest that transduction of MSG taste in the codling moth caterpillar relies mostly on cAMP-dependent signaling pathways.

Keywords: *Cydia pomonella*; Taste; Umami; Glutamate receptor; Feeding behavior; Apple

1. Introduction

Although larvae of the orchard pest, the codling moth [*Cydia pomonella* (L.)], are fruit eaters, adult females of the species lay most of their eggs on the leaves of trees to be infested, rather than on fruit. The newly hatched larvae, called neonates, search for fruit and upon finding the same, burrow into and feed within the fruit until their development is complete. However, the fact that neonates can feed and successfully molt to the second stage on foliage of several fruit trees (Tadic, 1957; Pszczolkowski et al., 2002a) suggests that feeding stimulants could be used as agents that would stimulate the neonates to feed on leaves before entering the fruit. Such agents could be mixed with commercial, orally active insecticide formulations, thus, either reducing the amount of toxic "hard pesticide" ingredients needed for effective pest control, or targeting pest caterpillars with insecticides that are environmentally friendly, but which need to be ingested in large amounts to exert desirable effects.

An umami substance, monosodium glutamate (MSG), a condiment used widely as a taste enhancer in the food industry, was proposed for use to enhance insecticide activity (Pszczolkowski and Brown, 2002; Pszczolkowski et al., 2002b). However, the rain-fastness of MSG was unsatisfactory for practical purposes (Pszczolkowski and Brown, 2002). In order to solve this latter problem we initiated a search for hydrophobic alternatives to MSG. As a part of this research, we pharmacologically characterized the glutamate receptors involved in perception of MSG by codling moth neonates (Pszczolkowski et al., 2003). We demonstrated that feeding on MSG-treated apple leaves is likely initiated via metabotropic glutamate receptors (mGluR) sensitive to (±)1-aminocyclopentane *trans*-1,3-dicarboxylic acid (*trans*-ACPD), a substance that has no effect on the quantity of leaf consumed. In contrast, consumption of MSG-treated foliage is stimulated with NMDA and external calcium (Pszczolkowski et al., 2003). The latter

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doi:10.1016/j.pbb.2005.11.008

finding led to another study in which we presented a hydrophobic alternative to MSG, a sparingly water soluble agonist of NMDAR, *trans*-1-aminocyclobutane-1,3-dicarboxylic acid (*trans*-ACBD) (Pszczolkowski and Brown, 2004); *trans*-ACBD increases leaf consumption and significantly also increases the efficacy of orally active insecticides. Moreover, the latter property of *trans*-ACBD is maintained in the field despite exposure to substantial rainfall (Pszczolkowski and Brown, 2004).

Confusion over the appropriate name for *trans*-ACBD that contributed to misinterpretation of literature data (Bell et al., 1980; Gaoni, 1988; Allan et al., 1990), along with the high price, discouraged the use of *trans*-ACBD in codling moth control. A less expensive compound that has the properties of *trans*-ACBD is needed, and to that end further studies on the pharmacology of leaf feeding by codling moth caterpillars are necessary.

Apart from the practical aspects, general studies on understanding MSG taste transduction are also needed. Thus far, a receptor believed to bind MSG as a taste stimulus has been identified in rat taste buds (Chaudhari et al., 1996, 2000). This receptor is similar to certain metabotropic glutamate receptors that are G-protein coupled and use a second messenger system for signal transduction. Specifically, this receptor is stimulated by L-(+)-2-amino-4-phosphonobutyric acid, L-AP4, a specific ligand for group III metabotropic glutamate receptors (Chaudhari et al., 1996), which binds to the metabotropic glutamate taste receptor, activates a G-protein (Ruiz et al., 2003), stimulates phosphodiesterase, and finally causes a decrease in intracellular cAMP (Chaudhari et al., 2000). It is unknown, however, how the decline in cAMP results in a receptor potential inside the taste cells. In vertebrates MSG may also be perceived by a broadly tuned L-amino acid sensor, a G-protein-coupled heterodimer belonging to the T1R family of mammalian taste receptors (Nelson et al., 2002; Zhao et al., 2003). Phospholipase C is postulated as a transducer for umami taste sensed by broadly tuned receptors of T1R family (Zhang et al., 2003).

Larval insects, during the process of probing the feeding substrate, use cone-shaped contact chemosensory receptors on their mouthparts. The tip of the "cone" has a pore through which the distal dendrites of the sensory neurons can contact the environment. During feeding, these dendrites are bathed by saliva and fluids from the food substrate and so respond to chemicals in solution (Chapman, 1998). Electrophysiological investigations of sensory cells are often used in insect taste studies; however, the minute size of codling moth neonates makes such analysis difficult, if not impossible (Pszczolkowski et al., 2003). Pharmacological analysis of feeding behavior, on the other hand, provides potential insight into molecular mechanisms that are likely involved in codling moth taste chemoreception (Pszczolkowski et al., 2003; Pszczolkowski and Brown, 2004). Surprisingly, the pharmacology of MSG taste perception in insects has been almost completely ignored, with the exception of recently initiated studies on codling moth neonates (Pszczolkowski et al., 2003). Both MSG and activators of vertebrate glutamate receptors stimulate commencement of or increase in feeding by codling moth neonates, in a manner similar to vertebrate response to glutamate (Pszczolkowski et al., 2003).

Data from the literature suggest that two additional well-defined groups of vertebrate mGluRs exist, both potentially involved in taste perception (Knopfel et al., 1995; Pin and Duvoisin, 1995; Roberts, 1995; Conn and Pin, 1997; Schoepp et al., 1999). Group I, described in rats and humans, is specifically activated by quisqualate, but also by (1S,3R)-1-aminocyclopentane-1,3-dicarboxylic acid, (1S,3R)-ACPD. The latter chemical is sometimes referred to as a general mGluR agonist. However, recent, comparative studies (Schoepp et al., 1999; Jane and Doherty, 2000) show that (1S,3R)-ACPD is the most potent group I agonist of mGluRs, and only moderately active as a group II agonist. The latter group has its own specific agonist (2R,4R)-4-aminopyrrolidine-2,4-dicarboxylate, (2R,4R)-APDC, which acts on mGluRs of group II with a 10–100-fold higher potency than (1S,3R)-ACPD (Schoepp et al., 1999). The signal transduction mechanisms involve activation of phospholipase C by group I and inactivation of adenylate cyclase by group II. mGluRs of group I and of group II are activated by *trans*-ACPD and, because *trans*-ACPD accelerates feeding commencement in codling moth neonates (Pszczolkowski et al., 2003), we speculate that either one of these two groups of mGluRs may be involved in MSG taste perception by neonates of this species.

The aim of the present study was to pharmacologically investigate how MSG induces feeding in codling moth neonates, thus, providing a background for further investigations of signal transduction during the process of feeding commencement. In particular, we studied how feeding commencement is altered by (1) a general agonist of vertebrate glutamate receptors, MSG, (2) vertebrate mGluR agonists that specifically stimulate either group I, group II, or group III of metabotropic glutamate receptors, (3) phospholipase C or phosphodiesterase inhibitors, (4) adenylate cyclase activator, and (5) combinations of mGluR agonists and pharmacological agents that alter signal transduction mechanisms.

2. Materials and methods

2.1. Insects and feeding substrate

Codling moths, originating from Wapato, WA, were reared at 25 °C, 70–80% RH under a L16/D8 light–dark photoregime, fed saturated sucrose solution, and allowed to lay eggs on wax paper. In all experiments, 0–0.5-h-old larvae were used.

Larvae were tested on apple leaves of Honeycrisp variety. New foliage (up to 3 days old) was used in all assays.

2.2. Chemicals

Metabotropic glutamate receptor agonists: (1S,3R)-1-aminocyclopentane-1,3-dicarboxylic acid, (1S,3R)-ACPD; (2R,4R)-4-aminopyrrolidine-2,4-dicarboxylate, (2R,4R)-APDC; and L-(+)-2-amino-4-phosphonobutyric acid, L-AP4; and chemicals affecting signal transduction: phospholipase C inhibitor, 1-[6-[[(17b)-3-methoxyestra-1,3,5(10)-trien-17-yl]amino]hexyl]-1H-pyrrole-2,5-dione, U 73122; adenylate cyclase

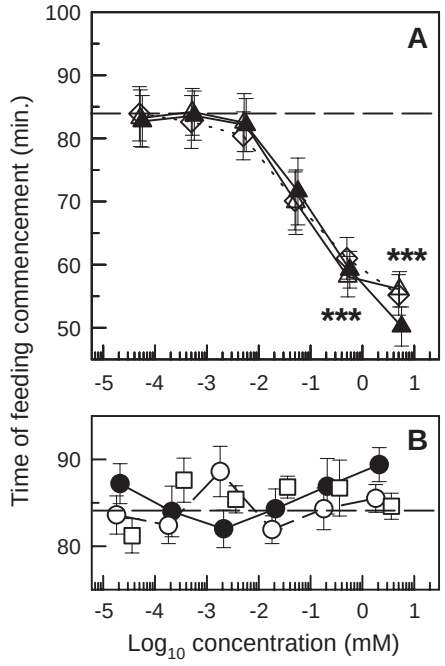

Fig. 1. A. Effects of mGluR agonists alone on feeding commencement by codling moth neonate larvae. Newly hatched neonates were allowed to feed on apple leaves treated with (1S,3R)-ACPD (solid triangles), (2R,4R)-APDC (open triangles), or L-AP4 (open diamonds). B. Effects of signal transduction modulators alone on feeding commencement by codling moth neonate larvae. Newly hatched neonates were allowed to feed on apple leaves treated with either phospholipase C inhibitor, U 73122, (solid circles), adenylate cyclase activator, NKH 477, (open circles) or phosphodiesterase inhibitor, Rolipram, (open squares). The average time of leaf consumption commencement by controls is 84.9±3.2 (horizontal dashed line showed on both panels). Each datum point shows mean±SEM corresponding to 64–72 larvae. ***$P<0.001$, Kruskal–Wallis test.

activator, *N*,*N*-dimethyl-(3R,4aR,5S,6aS,10S,10aR,10bS)-5-(acetyloxy)-3-ethenyldodecahydro-10,10b-dihydroxy-3,4a,7,7,10a-pentamethyl-1-oxo-1*H*-naphtho[2,1-b]pyran-6-yl ester b-alanine hydrochloride, NKH 477; and phosphodiesterase inhibitor, (4R)-4-[3-(Cyclopentyloxy)-4-methoxyphenyl]pyrrolidin-2-one, Rolipram were purchased from Tocris Cookson (Ellisville, MO). All other chemicals were purchased from Sigma (St. Louis, MO).

2.3. Feeding commencement bioassay

Leaf discs of uniform size (12 mm) were cut from leaves using a heavy-duty craft punch. Test solutions in 0.02% Triton X-100 in distilled water (10 μl) were applied on the upper surface of each leaf disc, distributed evenly, and the discs were allowed to air dry. The treated discs were mounted on glass microscope slides using a sandwich-like combination of double-sticky tape and self-adhesive three-ring binder reinforcement labels with a circular opening of 6 mm. Each bioassay station contained one section of leaf infested with one neonate under a glass cover slip and observed to record feeding activity. Additional details of this procedure are given elsewhere (Pszczolkowski and Brown, 2002, 2003, 2004). Each larva was observed for commencement of feeding at 15-min intervals for 3 h following infestation.

2.4. Experimental design

To delineate which classes of mGluRs are likely involved in perception of umami taste, concentration-dependent response of larvae to three mGluR agonists were studied in separate experiments. To obtain each concentration–response curve, leaf sections were treated with various concentrations of (1S,3R)-ACPD, (2R,4R)-APDC, or L-AP4. Control larvae were exposed to leaves treated with 10 μl 0.02% Triton X-100 only. On the basis of these experiments, standard 5 mM concentrations of respective mGluR agonists were selected to be used in further studies.

To identify putative signal transduction mechanisms, larvae were exposed to standard, 5 mM concentration of respective mGluR agonist in combination with various concentrations of signal transduction modulators specific or non-specific to particular class of metabotropic glutamate receptors under consideration. The following chemicals were used here: phospholipase C inhibitor, U 73122, adenylate cyclase activator, NKH 477, and phosphodiesterase inhibitor, Rolipram. There were two control groups here: a group exposed to leaves treated with 5 mM concentration of respective mGluR agonist, and a group exposed to various concentrations of U 73122, NKH 477, or Rolipram alone.

In the last experiment, possible relative contributions by particular mGluR-coupled signaling pathways to perceiving the signal from MSG were studied. Neonate larvae were exposed to various concentrations of signal transduction modulators in combination with 1 mM MSG. Control larvae were exposed to leaves treated with 1 mM MSG.

2.5. Statistics

None of datasets passed normality tests ($P<0.05$, GraphPad InStat, GraphPad Software, San Diego, CA). Therefore, mean times of feeding initiation±SEM were compared with Kruskal–Wallis test among control and experimental groups.

3. Results

3.1. Effects of mGluR agonists alone

All mGluR agonists accelerated commencement of feeding, and showed similar dynamics (Fig. 1A). Experimental larvae started to feed significantly earlier in presence of (1S,3R)-ACPD, (2R,4R)-APDC, or L-AP4 at concentrations as low as 0.5 mM ($P<0.001$, Kruskal–Wallis test). Control larvae started to feed 84.9±3.2 min after exposure. Average times of feeding commencement were reduced from about 85 to about 50 min in the presence of all three mGluR agonist.

3.2. Effects of signal transduction modulators alone

None of the signal transduction modulators tested (phospholipase C inhibitor U 73122, adenylate cyclase activator NKH 477, or phosphodiesterase inhibitor Rolipram) affected feeding commencement ($P>0.05$, Kruskal–Wallis test). The average time for commencement of feeding ranged from 81.2±2.72 min (3.6 mM Rolipram) to 89.4±1.96 min

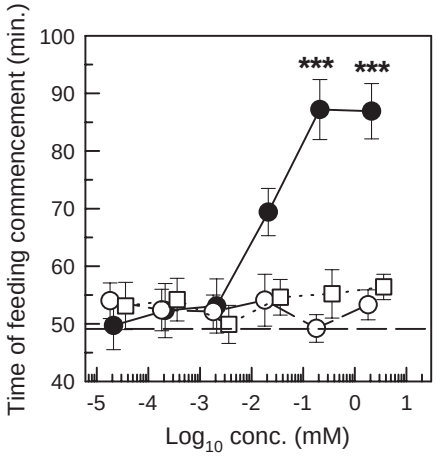

Fig. 2. Effects of signal transduction modulators on group I mGluRs agonist-induced feeding commencement by codling moth neonate larvae. Newly hatched neonates were allowed to feed on apple leaves concurrently treated with (1S,3R)-ACPD and either phospholipase C inhibitor, U 73122, (solid circles), adenylate cyclase activator, NKH 477, (open circles) or phosphodiesterase inhibitor, Rolipram, (open squares). The average time of leaf consumption commencement by (1S,3R)-ACPD-treated controls is 49.7±3.6 min (horizontal dashed line). Each datum point shows mean±SEM corresponding to 64–72 larvae. ***$P<0.001$, Kruskal–Wallis test.

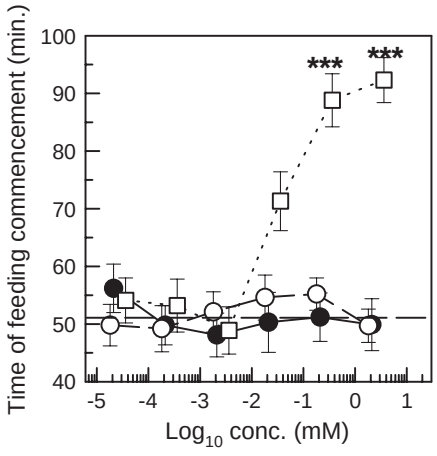

Fig. 4. Effects of signal transduction modulators on group III mGluRs agonist-induced feeding commencement by codling moth neonate larvae. Newly hatched neonates were allowed to feed on apple leaves concurrently treated with (1S,3R)-ACPD and either phospholipase C inhibitor, U 73122, (solid circles), adenylate cyclase activator, NKH 477, (open circles) or phosphodiesterase inhibitor, Rolipram, (open squares). The average time of leaf consumption commencement by L-AP4-treated controls is 51.7±3.3 min (horizontal dashed line). Each datum point shows mean±SEM corresponding to 64–72 larvae. ***$P<0.001$, Kruskal–Wallis test.

(2.1×10^{-4}M NKH 477) (Fig. 1B), which was similar to that of control neonates exposed to solvent only ($P>0.05$, Kruskal–Wallis test, see above).

3.3. Effects of signal transduction modulators on (1S,3R)-ACPD-induced feeding commencement

In response to 5 mM (1S,3R)-ACPD (agonist of group I mGluR) alone the neonates started to feed 49.7±3.6 min after exposure. The phospholipase C inhibitor, U 73122, abolished feeding stimulatory effects of (1S,3R)-ACPD; the average time for commencement increased with increasing concentrations, at maximum of 0.2–2 mM (Fig. 2, $P<0.001$, Kruskal–Wallis test). In contrast, the adenylate cyclase activator (NKH 477) and the phosphodiesterase inhibitor (Rolipram) did not suppress the stimulatory action of (1S,3R)-ACPD ($P>0.05$, Kruskal–Wallis test).

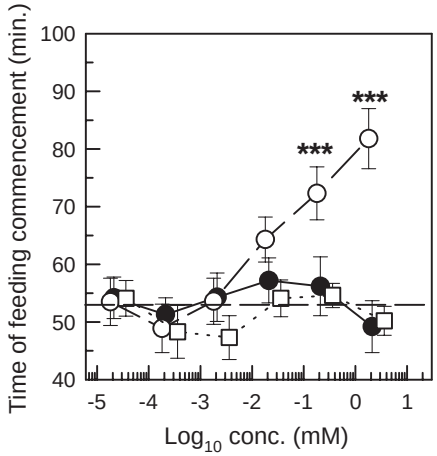

Fig. 3. Effects of signal transduction modulators on group II mGluRs agonist-induced feeding commencement by codling moth neonate larvae. Newly hatched neonates were allowed to feed on apple leaves concurrently treated with (1S,3R)-ACPD and either phospholipase C inhibitor, U 73122, (solid circles), adenylate cyclase activator, NKH 477, (open circles) or phosphodiesterase inhibitor, Rolipram, (open squares). The average time of leaf consumption commencement by (2R,4R)-APDC-treated controls is 53.0±3.8 min (horizontal dashed line). Each datum point shows mean±SEM corresponding to 64–72 larvae. ***$P<0.001$, Kruskal–Wallis test.

3.4. Effects of signal transduction modulators on (2R,4R)-APDC-induced feeding commencement

(2R,4R)-APDC, an agonist of group II mGluR accelerated feeding commencement when used alone at 5 mM; the neonates started to feed 53.0±3.8 min after exposure. Increasing concentrations of adenylate cyclase activator, NKH 477, resulted in concentration-dependent increase of average time for feeding commencement (Fig. 3, $P<0.001$, Kruskal–Wallis test). However, action of (2R,4R)-APDC on feeding commencement remained unaffected by either the phospholipase C or phosphodiesterase inhibitor ($P>0.05$, Kruskal–Wallis test).

3.5. Effects of signal transduction modulators on L-AP4-induced feeding commencement

L-AP4, an agonist of group III mGluR, also accelerated commencement of feeding at 5 mM concentration. Neonates started to feed 51.7±3.3 min after exposure. Feeding-stimulatory effects of L-AP4 disappeared in the presence of the phosphodiesterase inhibitor, Rolipram, which increased average time for commencement of feeding with increasing concentrations. Maximum effects of Rolipram were observed at 0.4 and 4 mM concentrations (Fig. 4, $P<0.001$, Kruskal–Wallis test). The adenylate cyclase activator and phospholipase

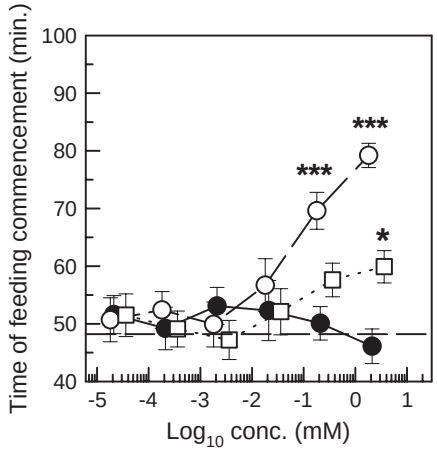

Fig. 5. Effects of signal transduction modulators on MSG-induced feeding commencement by codling moth neonate larvae. Newly hatched neonates were allowed to feed on apple leaves concurrently treated with (1S,3R)-ACPD and either phospholipase C inhibitor, U 73122, (solid circles), adenylate cyclase activator, NKH 477, (open circles) or phosphodiesterase inhibitor, Rolipram, (open squares). The average time of leaf consumption commencement by MSG-treated controls is 48.2±3.2 min (horizontal dashed line). Each datum point shows mean±SEM corresponding to 64–72 larvae. ***$P<0.001$, *$P<0.05$, Kruskal–Wallis test.

C inhibitor did not alter feeding stimulatory effects of L-AP4 ($P>0.05$, Kruskal–Wallis test).

3.6. Effects of signal transduction modulators on MSG-induced feeding commencement

MSG (1 mM concentration) accelerated feeding commencement; the neonates started to feed 48.2±3.2 min after exposure. This stimulatory effect was maintained in the presence of the phospholipase C inhibitor, U 73122, alone (Fig. 5, $P>0.05$, Kruskal–Wallis test). The phosphodiesterase inhibitor, Rolipram (3 mM), abolished the stimulatory effect of MSG, and delayed average time for commencement of feeding by approximately 10 min (Fig. 5, $P<0.05$, Kruskal–Wallis test). The adenylate cyclase activator, NKH 477, was more effective in reversing stimulatory effects: 0.1–1 mM significantly increased the average time of feeding commencement (Fig. 5, $P<0.001$, Kruskal–Wallis test).

4. Discussion

This study extends our general knowledge of the mechanisms that regulate insect-feeding behavior. Three different metabotropic glutamate receptors agonists, (1S,3R)-ACPD, (2R,4R)-APDC, and L-AP4 accelerate commencement of leaf feeding by codling moth neonates. The activity of these metabotropic glutamate receptor agonists is modulated by specific signal transduction modulators. The phospholipase C inhibitor, U 73122, reverses the feeding stimulatory effects of (1S,3R)-ACPD, whereas the adenylate cyclase activator, NKH 477, affects (2R,4R)-APDC, and the phosphodiesterase inhibitor, Rolipram, abolishes stimulatory effects of L-AP4 only. The feeding-stimulatory effects of the general glutamate receptor agonist MSG are abolished by NKH 477 and Rolipram, but not by U 73122.

4.1. Effects of metabotropic glutamate receptor agonists and signal transduction modulators

Feeding in codling moth larvae is induced by three different mGluR agonists known to specifically stimulate different groups of vertebrate metabotropic glutamate receptors: group I responding to (1S,3R)-ACPD, group II activated by (2R,4R)-APDC, and group III, stimulated by L-AP4. This finding corroborates previous findings with this insect and with vertebrates. It has been shown that feeding in codling moth larvae could be induced by both MSG (Pszczolkowski et al., 2002b) and *trans*-ACPD (Pszczolkowski et al., 2003), the latter being a general agonist that stimulates both group I and group II mGluRs in vertebrate systems (Pin and Duvoisin, 1995; Knopfel et al., 1995; Harris et al., 2002). On the other hand, it has been demonstrated that metabotropic glutamate receptors present in rat lingual epithelium respond to L-AP4, and that this compound mimics the taste of MSG in behavioral tests performed in rats and humans (Chaudhari et al., 1996; Kurihara and Kashiwayanagi, 1998; Sako and Yamamoto, 1999; Delay et al., 2000; Chaudhari et al., 2000). In light of our current results, it appears that the receptors used for probing the feeding substrate in codling moth neonates share pharmacological characteristics with those found in vertebrates (group III mGluRs). However, leaf consumption in the caterpillar is also initiated by mGluR agonists that stimulate groups I and II of vertebrate metabotropic receptors. This finding appears to indicate a unique set of glutamate receptors involved in taste reception that are specific to codling moth larvae, and have not been reported for vertebrates in the context of taste.

The experiments on the effects of specific mGluR agonists combined with drugs specifically affecting signal transduction mechanisms suggest that each tested agonist of metabotropic glutamate receptors uses a different signaling pathway as the insect probes the leaf surface as a potential feeding substrate. The phospholipase C inhibitor (U 73122) abolished stimulatory effects of (1S,3R)-ACPD on feeding in a dose-dependent manner, but did not affect acceleration of feeding commencement by the other two mGluR agonists. Similarly, the adenylate cyclase activator (NKH 477) specifically abolished stimulatory effects of (2R,4R)-APDC and the phosphodiesterase inhibitor (Rolipram) abolished stimulatory effects of L-AP4 only.

The above findings are in concordance with the current knowledge of vertebrate mGluR pharmacology, which divides mGlu receptors into three distinct groups (Knopfel et al., 1995; Pin and Duvoisin, 1995; Roberts, 1995; Conn and Pin, 1997; Schoepp et al., 1999). Group I comprises two subclasses, $mGluR_1$ and $mGluR_5$, both of which are sensitive to (1S,3R)-ACPD, and both use phospholipase C for signal transduction. Group II, consisting of two subclasses, $mGluR_2$ and $mGluR_3$, is specifically sensitive to (2R,4R)-APDC and signals through decrease of cAMP levels via decreased activity of adenylate cyclase. Finally, responses to three subclasses of group III ($mGluR_4$, $mGluR_6$, and $mGluR_8$) are elicited by L-AP4, which decreases cAMP levels (Knopfel et

Table 1
Putative, glutamate receptor-coupled signaling pathways that codling moth neonate larvae probably use in the process of feeding commencement

Putative signaling pathway	1	2	3
Specific agonist	(1S,3R)-ACPD	(2R,4R)-APDC	L-AP4
Receptor	mGluR I	mGluR II	mGluR III
Primary transduction mechanism	↑ PLC	↓ AC	↑ PDE
Secondary transduction mechanism	↓ cAMP	↓ cAMP	

Pathways 2 and 3 are likely dominant in neonate response to monosodium glutamate.
↑ PLC: Phospholipase C increase, ↓ AC: Adenylate cyclase decrease, ↑ PDE: Phosphodiesterase increase, ↓ cAMP: cyclic AMP decrease, ?: unknown process (processes).

al., 1995; Pin and Duvoisin, 1995; Roberts, 1995; Conn and Pin, 1997; Schoepp et al., 1999). More recent reports suggest that transduction mechanisms may vary in group III, in terms of dependence on species or site of the receptors' expression. For instance, in rat microglia all aforementioned receptor subclasses, including $mGluR_4$, signal via inhibition of adenylate cyclase activity (Taylor et al., 2003). However, in rat lingual tissue, phosphodiesterase-based system of signaling from $mGluR_4$ is postulated (Roper, 2002). The group III mGluRs localized in mouse striatum have been shown to signal through phosphodiesterase not via adenylate cyclase (Dohovics et al., 2003).

On the basis of our experimental work and reports in the literature, we suggest that the receptors and signaling pathways involved in MSG-evoked commencement of feeding by codling moth neonates, on the one hand, share similarity with vertebrate umami taste perception systems, but on the other, appear to use some unique transduction mechanisms that have not been demonstrated or postulated in vertebrates. Stimulatory effects of L-AP4 and inhibitory effects of concurrently applied phosphodiesterase inhibitor, Rolipram, correspond with a model of umami taste perception presented by Roper (2002), Chaudhari et al. (1996, 2000) and Abaffy et al. (2003) for the rat and that of Kurihara and Kashiwayanagi (1998) for human. Inhibitory effects of phospholipase C inhibitor corroborate the report of Zhang et al. (2003), who suggest common molecular mechanisms for sweet, bitter, and umami taste transduction in mice, phospholipase C actually being a common transducing element. However, in the case of codling moth neonates the feeding stimulatory effects of (1S,3R)-ACPD and (2R,4R)-APDC, and reversal of these effects by adenylate cyclase activator, NKH 477, suggest alternative pathways of MSG perception, perhaps specific to insects. In sum, it seems that codling moth neonates may perceive MSG not only via group III mGluRs coupled to phosphodiesterase signaling system (as in vertebrates), but also via mGluRs of group I signaling through phospholipase C, and via mGluRs of group II that signal through adenylate cyclase.

4.2. Effects of signal transduction modulators on feeding induction by MSG

Our results shown in Fig. 5 suggest that MSG likely uses only two (out of the three potential) signaling pathways. The two pathways responding to MSG in the process of feeding commencement differ at their initial steps; one likely depends on adenylate cyclase, whereas the other likely uses phosphodiesterase (Table 1). Both pathways likely converge downstream, to ultimately respond to MSG by decrease in cAMP concentrations (Table 1). If so, it is reasonable to hypothesize that MSG induces feeding via group II and III metabotropic glutamate receptors. Why MSG does not appear to signal through phospholipase C (and presumably via group I mGluRs) remains an open question, the answer to which will require further experimentation. Perhaps, different metabotropic glutamate receptors involved in taste perception by codling moth neonates bind MSG with different affinity, or different signaling pathways transduce umami taste with differential efficacy.

4.3. General implications of our study

Our report suggests that in codling moth neonate several different glutamate receptors and corresponding transduction systems may coexist in one organism complementing each other in one behavioral task, the process of probing the leaf surface as a potential feeding substrate. Because various mGluR ligands alter feeding initiation only (Pszczolkowski et al., 2003; this paper), a possibility that observed changes in feeding behavior are post-ingestive effects (e.g. influencing food ingestion control system and increasing the need for food) should be excluded. Instead, mGluRs ligands appear to induce a peripheral chemosensory response in codling moth neonates before feeding actually starts. Taste perception likely plays a role here, perhaps a dominant role, as it has been observed in other insect species (Chapman, 1998). Therefore, our hypothesis about multiple transduction pathways involved in codling moth neonate response to MSG must be viewed in light of a contentious issue in basic research on vertebrate taste: the issue of two, mutually exclusive models of taste perception. The first model (model A) proposes that taste is perceived by a large fraction of broadly tuned taste receptor cells, where many cells can express multiple receptor types and multiple signaling pathways, even within the same taste modality (Caicedo et al., 2002; Margolskee, 2002). The alternative model (model B) favors the idea that each taste receptor cell is dedicated to a single taste quality, but all cells use the same signaling cascade (Adler et al., 2000; Nelson et al., 2001; Amrein and Bray, 2003), which has been supported by experiments of Zhang et al. (2003).

In some insects, each taste cell responds to a different class of compounds (Chapman, 1998), which suggests that model B may be operational. On the other hand, some caterpillars (e.g., 7those of *Gramma geneura*) possess broadly tuned taste neurons responding to sugars, amino acids, and glycosides (Bernays and Chapman, 2001). In caterpillars of the tobacco hawkmoth, *Manduca sexta*, one specific chemoreceptor cell may respond to different extracts obtained from the same plant, using different solvents (i.e., to presumably different chemicals) (Peterson et al., 1993). Similar effects of pyrrolizidine alkaloids in yet another caterpillar, *Utetheisa ornatrix* (Bernays

et al., 2003), suggest that model A better illustrates insect taste mechanisms, at least in relation to some sapid molecules from plants. The present results suggest that some elements of both models may be proposed for MSG perception in one organism, the codling moth caterpillar. At present, we do not know whether the same dendrite responds to the three different mGluR ligands in this insect. However, we have shown that three different glutamate mimics may signal in the same organism through three different signaling pathways (Figs. 2–4, Table 1). Moreover, MSG may use two different signaling pathways depending either on adenylate cyclase or phosphodiesterase (Fig. 5, Table 1). Therefore, it appears that in codling moth neonates there are multiple signaling pathways within the same chemoreception modality, an element resembling that in model A for vertebrate taste perception. Additionally, similar to vertebrate systems (Taylor et al., 2003, Dohovics et al., 2003), it is reasonable to suggest that both adenylate cyclase- and phosphodiesterase-dependent pathways of MSG taste transduction converge to a common cAMP-dependent cascade. If the latter is true, the MSG taste transduction in codling moth neonates may have a model B feature; a commonality of signaling pathways, albeit downstream of the signaling pathway. We realize that such a hypothesis is highly speculative at the present stage of our studies on codling moth chemoreception. However, the pharmacological analysis presented in this paper delineates directions for further studies on cellular responses to MSG in the process of feeding substrate probing by codling moth neonates. For the reasons mentioned in the Introduction, such directions could not have been delineated for codling moth neonates by means such as measuring electrophysiological responses of single taste neurons. The search for alternative methods that will be useful for studying MSG taste transduction in taste cells of codling moth larvae is under way in our laboratory. One such method could be investigating whether or not older larvae (in which electrophysiological methods may be applied as the individuals are significantly larger) would respond to MSG in a manner similar to neonates. If so, measuring electrophysiological responses of single taste neurons to MSG or group specific mGluR agonists will allow us empirically test the hypothesis of MSG perception by codling moth larvae.

4.4. Practical prospects of our study

As mentioned earlier, an inexpensive rain-fast feeding stimulant is needed for more effective and rational control of codling moth caterpillars, a serious pest of apple production. It has been reported previously (Pszczolkowski et al., 2003; Pszczolkowski and Brown, 2004), that larval feeding in codling moth is controlled by two sets of glutamate receptors: metabotropic glutamate receptors control commencement of feeding and NMDA receptors control feeding continuation throughout 24 or 48 h of continuous exposure. In our present research, L-AP4 induced feeding in codling moth neonates. It is noteworthy that some structurally similar compounds, [e.g. 2-amino-phosphono acids (Evans et al., 1982) or dicarboxylic amino acids (Cotman et al., 2004)] are known to affect function of NMDA receptors. We believe that research on the potential of these compounds to increase leaf consumption is needed, as a rain-fast alternative to MSG that would be less expensive or easier to manufacture than the recently proposed *trans*-ACBD (Pszczolkowski and Brown, 2004).

Acknowledgements

This work was partially supported by Washington State Tree Fruit Research Commission. This is contribution No. 05-282-J from the Kansas Agricultural Experiment Station, Manhattan, Kansas.

References

Abaffy T, Trubey KR, Chaudhari N. Adenylyl cyclase expression and modulation of cAMP in rat taste cells. Am J Physiol Cell Physiol 2003;284:C1420–8.

Adler E, Hoon MA, Mueller KL, Chandrashekar J, Ryba NJ, Zuker CS. A novel family of mammalian taste receptors. Cell 2000;100:693–702.

Allan RD, Hanrahan JR, Hambley TW, Johnston GA, Mewett KN, Mitrovic AD. Synthesis and activity of a potent *N*-methyl-D-aspartic acid agonist, *trans*-1-aminocyclobutane-1,3-dicarboxylic acid, and related phosphonic and carboxylic acids. J Med Chem 1990;33:2905–15.

Amrein H, Bray S. Bitter-sweet solution in taste transduction. Cell 2003; 112:283–4.

Bell EA, Qureshi MY, Pryce RJ, Janzen DH, Lemke P, Clardy J. 2,4-methanoproline (2-carboxy-2,4-methanopyrrolidine) and 2,4-methanoglutamic acid (1-amino-1,3-dicarboxycyclobutane) in seeds of *Ateleia herbert-smithii* Pittier (Leguminosae). J Am Chem Soc 1980;102:1409–12.

Bernays EA, Chapman RF. Taste cell responses in the polyphagous arctiid, *Grammia geneura*: towards a general pattern for caterpillars. J Insect Physiol 2001;47:1029–43.

Bernays EA, Chapman RF, Lamunyon CW, Hartmann T. Taste receptors for pyrrolizidine alkaloids in a monophagous caterpillar. J Chem Ecol 2003;29:1709–22.

Caicedo A, Kim KN, Roper SD. Individual mouse taste cells respond to multiple chemical stimuli. J Physiol 2002;544:501–9.

Chapman RF. The insects. Structure and function. Cambridge: Cambridge University Press; 1998. p. 645–51.

Chaudhari N, Yang H, Lamp C, Delay E, Cartford C, Than T, et al. The taste of monosodium glutamate: membrane receptors in taste buds. J Neurosci 1996;16:3817–26.

Chaudhari N, Landin AM, Roper SD. A metabotropic glutamate receptor variant functions as a taste receptor. Nat Neurosci 2000;3:113–9.

Conn PJ, Pin JP. Pharmacology and functions of metabotropic glutamate receptors. Annu Rev Pharmacol Toxicol 1997;37:205–37.

Cotman CW, Kahle JS, Miller SM, Ulas J, Bridges RJ. Excitatory amino acid neurotransmission. Psychopharmacology- the fourth generation of progress. American College of Neuropsychopharmacology; 2004. http://www.acnp.org/content-32.html.

Delay ER, Beaver AJ, Wagner KA, Stapleton JR, Harbaugh JO, Catron KD, et al. Taste preference synergy between glutamate receptor agonists and inosine monophosphate in rats. Chem Senses 2000;25:507–15.

Dohovics R, Janaky R, Varga V, Saransaari P, Oja SS. Cyclic AMP-mediated regulation of striatal glutamate release: interactions of presynaptic ligand- and voltage-gated ion channels and G-protein-coupled receptors. Neurochem Int 2003;43:425–30.

Evans RH, Francis AA, Jones AW, Smith DA, Watkins JC. The effects of a series of omega-phosphonic alpha-carboxylic amino acids on electrically evoked and excitant amino acid-induced responses in isolated spinal cord preparations. Br J Pharmacol 1982;75:65–75.

Gaoni Y. Regiospecific additions of hydrazoid acid and benzylamine to 1-(arylosulfonyl)bicycle(110) butanes Application to the synthesis of *cis* and *trans* 2,7-methanoglutamic acids. Tetraherdon Lett 1988;29:1591–4.

Harris BR, Prendergast MA, Gibson DA, Rogers DT, Blanchard JA, Holley RC, et al. Acamprosate inhibits the binding and neurotoxic effects of *trans*-ACPD, suggesting a novel site of action at metabotropic glutamate receptors. Alcohol Clin Exp Res 2002;2:1779–93.

Jane DE, Doherty AJ. Muddling trough the mGlu maze? Tocris reviews; 2000. No. 13.

Knopfel T, Kuhn R, Allgeier H. Metabotropic glutamate receptors: novel targets for drug development. J Med Chem 1995;38:1417–26.

Kurihara K, Kashiwayanagi M. Introductory remarks on umami taste. Ann NY Acad Sci 1998;855:393–7.

Margolskee RF. Molecular mechanisms of bitter and sweet taste transduction. J Biol Chem 2002;277:1–4.

Nelson G, Hoon MA, Chandrashekar J, Zhang Y, Ryba NJ, Zuker CS. Mammalian sweet taste receptors. Cell 2001;106:381–90.

Nelson G, Chandrashekar J, Hoon MA, Feng L, Zhao G, Ryba NJ, et al. An amino-acid taste receptor. Nature 2002;416:199–202.

Peterson SC, Hanson FE, Warthen Jr JD. Deterrence coding by a larval *Manduca* chemosensory neurone mediating rejection of a non-host plant, *Canna generalis* L. Physiol Entomol 1993;18:285–95.

Pin JP, Duvoisin R. The metabotropic glutamate receptors: structure and functions. Neuropharmacology 1995;34:1–26.

Pszczolkowski MA, Brown JJ. Prospects of monosodium glutamate use for enhancement of pesticides toxicity against the codling moth. Phytoparasitca 2002;30:243–52.

Pszczolkowski MA, Brown JJ. Effects of sugars and non-nutritive sugar substitutes on consumption of apple leaves by neonates of codling moth. Phytoparasitica 2003;31:283–91.

Pszczolkowski MA, Brown JJ. Enhancement of spinosad toxicity to *Cydia pomonella* neonates by monosodium glutamate receptor agonist. Phytoparasitica 2004;32:342–50.

Pszczolkowski MA, Matos LF, Brown R, Brown JJ. Feeding and development of codling moth larvae on apple leaves. Ann Entomol Soc Am 2002a; 95:603–7.

Pszczolkowski MA, Matos L, Zahand A, Brown JJ. Effect of monosodium glutamate on apple leaf consumption by codling moth larvae. Entomol Exp Appl 2002b;103:91–8.

Pszczolkowski MA, Zahand A, Bushman SM, Brown JJ. Effects of calcium and glutamate receptor antagonists on leaf consumption by lepidopteran neonates. Pharmacol Biochem Behav 2003;74:389–94.

Roberts PJ. Pharmacological tools for the investigation of metabotropic glutamate receptors (mGluRs): phenylglycine derivatives and other selective antagonists—an update. Neuropharmacology 1995;34:813–9.

Roper SD. Taste: cellular basis. Encyclopedia of life sciences, vol. 18. London: Macmillan Publishers Ltd.; 2002. p. 117–24.

Ruiz CJ, Wray K, Delay E, Margolskee RF, Kinnamon SC. Behavioral evidence for a role of alpha-gustducin in glutamate taste. Chem Senses 2003;28:573–9.

Sako N, Yamamoto T. Analyses of taste nerve responses with special reference to possible receptor mechanisms of umami taste in the rat. Neurosci Lett 1999;261:109–12.

Schoepp DD, Jane DE, Monn JA. Pharmacological agents acting at subtypes of metabotropic glutamate receptors. Neuropharmacology 1999;38: 1431–76.

Tadic M, 1957. Jabucni smotavac, biologija kao osnova za njegovo suzbitanje, Universitet u Beogradu, Beograd; 1957. p. 40–6.

Taylor DL, Diemel LT, Pocock JM. Activation of microglial group III metabotropic glutamate receptors protects neurons against microglial neurotoxicity. J Neurosci 2003;23:2150–60.

Zhang Y, Hoon MA, Chandrashekar J, Mueller KL, Cook B, Wu D, et al. Coding of sweet, bitter, and umami tastes: different receptor cells sharing similar signaling pathways. Cell 2003;112:293–301.

Zhao GQ, Zhang Y, Hoon MA, Chandrashekar J, Erlenbach I, Ryba NJ, et al. The receptors for mammalian sweet and umami taste. Cell 2003;115: 255–66.