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Pharmacological properties of L-glutamate receptors associated with the crayfish hindgut

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Abstract Pharmacological agents were used to characterize glutamate receptors associated with crayfish hindgut. L-Glutamate reliably increased tonus in isolated hindguts of *Procambarus clarkii* and suppressed spontaneous hindgut contractions at concentrations of 10 μmol l^{-1} or higher. Quisqualate and ibotenate mimicked the effects of L-glutamate. Experiments with strips and rings of hindgut tissue indicate that glutamate acts on both circular and longitudinal muscles. Hindgut contractions were not affected by (±)-α-amino-3-hydroxy-5-methyl-isoxazole-4-propionoic acid, *N*-methyl-D-aspartate, domoate or the metabotropic glutamate receptor agonist, (1S,3R)-1-amino-1-carboxycyclopentane-3-acetic acid. Picrotoxin, at 50 μmol l^{-1}, did not alter the ibotenate-induced reduction in contraction frequency, suggesting that this effect is not produced by inhibitory glutamate receptors. The glutamate-induced increase in tonus was antagonized by Joro spider toxin, JSTX-3. Thus, glutamate receptors associated with crayfish hindgut muscles are of the quisqualate type but are also sensitive to ibotenate. Elevating extracellular potassium concentration mimicked all of the effects of glutamate, suggesting that excessive depolarization may contribute to the suppression of contractions at high agonist concentrations.

Keywords Crustacean hindgut · Glutamate receptors · Ibotenate · Quisqualate

Abbreviations *AMPA* (±)-α-amino-3-hydroxy-5-methyl-isoxazole-4-propionoic acid · *Homo-ACPD* (1S,3R)-1-amino-1-carboxycyclopentane-3-acetic acid · *JSTX-3* Joro spider toxin · *NMDA N*-methyl-D-aspartate

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Introduction

Peristalsis in the hindgut of decapod crustaceans involves circular and longitudinal muscles, whose contractions are enhanced and coordinated by the central nervous system (Wales 1983; Winlow and Laverack 1972a, 1972b, 1972c). The motor output to the hindgut is carried by axons in the 7th abdominal roots. Nearly all of these axons have their cell bodies in the 6th abdominal ganglion, but a few can be traced to cell bodies in more anterior ganglia (Kondoh and Hisada 1986; Mercier et al. 1991; Muramoto 1977). The axons supplying the hindgut give rise to an inner plexus and an outer plexus, each containing many fine branches and nerve endings (Alexandrowicz 1909; Elofsson et al. 1968). Extracts of crustacean hindguts have been found to contain dopamine (Elekes et al. 1988; Mercier et al. 1991), orcokinin (sequence: NFDEIDRSGFGFN; Bungart et al. 1994), proctolin (RYLPG-OH; Mercier et al. 1997) and an FMRFamide-like peptide (Mercier et al. 1997). All of these putative transmitters excite hindgut muscles, but their roles in controlling peristalsis have not been firmly established.

Glutamate is the most common excitatory neurotransmitter at neuromuscular synapses of crustaceans (Atwood 1982; Takeuchi and Takeuchi 1964) and insects (Usherwood 1967; Usherwood et al. 1968), and its function as a transmitter has been studied very thoroughly using exoskeletal muscles. The postsynaptic glutamate receptors of arthropods do not respond to *N*-methyl-D-aspartic acid (NMDA) or α-amino-3-hydroxy-5-methylisoxazole-4-propanoic acid (AMPA), but they are activated by quisqualate (Usherwood and Blagbrough 1992). Aside from two early reports (Florey 1961; Jones 1962) a neurotransmitter role for glutamate in the crustacean hindgut has been largely unexplored. Florey (1961) reported that glutamate (at approximately 30 μmol l^{-1}) inhibited contractions of hindguts from three crayfish species (*P. clarkii, Orconectes virilis and Pacifastacus leniusculus*). However, Jones (1962) showed

that glutamate (at concentrations comparable to those used by Florey) stimulates contractions of hindguts from the crayfish, *Astacus astacus*, and suggested that Florey's results might reflect species differences. Jones (1962) also showed that L-glutamate is ten times more potent than D-glutamate, but that both isomers elicit excitatory actions. The issue of whether glutamate's role in crustacean hindgut muscles is primarily excitatory or inhibitory has not been pursued.

There is considerable evidence to support a neurotransmitter role for L-glutamate in insect hindguts (Holman and Cook 1970; Cook and Holman 1979a, 1979b; Dunbar and Piek 1983; Kits et al. 1984). One pharmacological study (Izawa et al. 1988) characterized three glutamate receptor sub-types in insect hindgut: (1) activated by quisqualate and mediating phasic and tonic contractions, (2) activated by kainate and domoate, eliciting only phasic contractions, and (3) activated by ibotenate, inhibiting contractions. The inhibitory effects of ibotenate are mediated by inhibitory glutamate receptors (IGluRs), permeable to Cl^- ions (Cleland 1996).

The aims of the present work were to re-examine the effects of L-glutamate on the crayfish hindgut and to characterize the glutamate receptors pharmacologically. The present study shows that L-glutamate and two other glutamate-receptor agonists induce slow contractures in isolated hindguts of *P. clarkii*. High concentrations of all three agonists suppress spontaneous gut contractions, and this may be attributed to receptor desensitization or excessive depolarization. Thus, in general, the present results support the conclusion of Jones (1962) that the effects of L-glutamate on crayfish hindgut are excitatory. The pharmacological properties of the glutamate receptors associated with crayfish hindgut are similar to those of other arthropod glutamate receptors.

Materials and methods

Adult crayfish (*P. clarkii*) from Atchafalaya Biological Supply (Raceland, La., USA) were maintained in tanks of aerated freshwater at 15°C on a diet of Tender Vittles cat food. The abdomen was removed, the dorsal abdominal shell was dissected away, and the entire abdominal portion of the hindgut was isolated and placed in a Petri dish lined with Sylgard and containing crayfish physiological saline (van Harreveld 1936) with the following composition (mmol l^{-1}): NaCl, 205; KCl, 5.3; $CaCl_2$, 13.5; $MgCl_2$, 2.45; HEPES, 5; pH 7.4. Contractions were recorded in a chamber with a volume of 0.5 ml. The chamber was perfused continuously with crayfish saline at a rate of 3.0 ml min^{-1} using a peristaltic pump to supply saline at one end and a vacuum pump to remove saline at the other end. Pharmacological agents were tested by changing solutions delivered to the recording chamber via the peristaltic pump. All experiments were carried out at 21°C.

Contractions were recorded using a Grass FT03 tension transducer connected to a Grass Model 7B polygraph. Small sections of hindgut, approximately 5–8 mm long, were placed in the recording dish and pinned at one end with a stainless steel minutien pin. The free end of the hindgut was then attached to a hooked pin, connected to the transducer by a 51-mm-long stainless steel dissecting probe. This arrangement amplified contractions by effectively extending the length of the spring. Care was taken not to stretch the tissue too much to avoid tearing.

In some experiments contractions were recorded preferentially from either circular or longitudinal muscles as described elsewhere (Mercier and Lee 2002). For the longitudinal muscles, a longitudinal strip of tissue was cut, isolating one of the six bands of longitudinal muscles. One end of the strip was pinned to the bottom of the recording dish, and the stylus from the force transducer was attached at 90° to the free end. For the circular muscles, the hindgut was cut transversely to produce a ring of tissue approximately 1–2 mm in diameter. A pin was then placed through the ring and secured in the bottom of the dish, and the pin attached to the transducer was placed through the ring at the other side and oriented at 90°.

Quisqualate, domoate, NMDA and AMPA were obtained from Sigma Chemical (St. Louis, Mo., USA). Ibotenate, Joro spider toxin (JSTX-3), (1S, 3R)-1-amino-1-carboxycyclopentane-3-acetic acid (homo-ACPD), (±)-trans-1-amino-1-carboxycyclopentane-2-acetic acid (ACPD), and picrotoxin were obtained from Calbiochem (LaJolla, Calif., USA). All other chemicals were obtained from British Drug House (Toronto, ON).

Results

L-Glutamate, quisqualate and ibotenate all affected hindgut contractions. All three of these glutamate agonists elicited slow contractions lasting approximately 30–60 s (Fig. 1). This effect, which was interpreted as an increase in tonus, typically occurred at a threshold concentration of 0.1–1 μmol l^{-1} for all three compounds. As in earlier reports (e.g., Florey 1961; Jones 1962; Mercier et al. 1991, 1997; Mercier and Lee 2002), the isolated crayfish hindguts generated spontaneous phasic contractions lasting about 1–2 s. All three glutamate agonists appeared to modulate these spontaneous contractions as well. In many preparations, the rise in tonus was accompanied by either a single phasic contraction or a brief burst of phasic contractions; these excitatory effects were more obvious in preparations with less spontaneous activity than in more active preparations (Fig. 1C). At concentrations of 10 μmol l^{-1} or higher, spontaneous contractions were usually suppressed, and in many cases they were completely suppressed at the highest concentrations tested (50 μmol l^{-1} to 1 mmol l^{-1}). All of the effects of the three agonists reversed completely when the preparations were washed with saline for 5–10 min.

Figures 2 and 3 illustrate the time course for effects of L-glutamate, quisqualate and ibotenate on frequency and amplitude of spontaneous contractions. These data were averaged from eight to nine preparations in each case. There appears to be a transient increase in contraction frequency within 30 s of treatment with 100 μmol l^{-1} L-glutamate, 10–100 μmol l^{-1} quisqualate and 50 μmol l^{-1} ibotenate. Quisqualate, at 10–100 μmol l^{-1}, appeared to increase contraction amplitude slightly at 30 s (Fig. 3); transient changes in amplitude within the first minute of application are less obvious for ibotenate and are not at all apparent for L-glutamate. One difficulty with this type of analysis is that the apparent effects depended to some extent on the amount of spontaneous activity, which varied considerably among different preparations. Less active

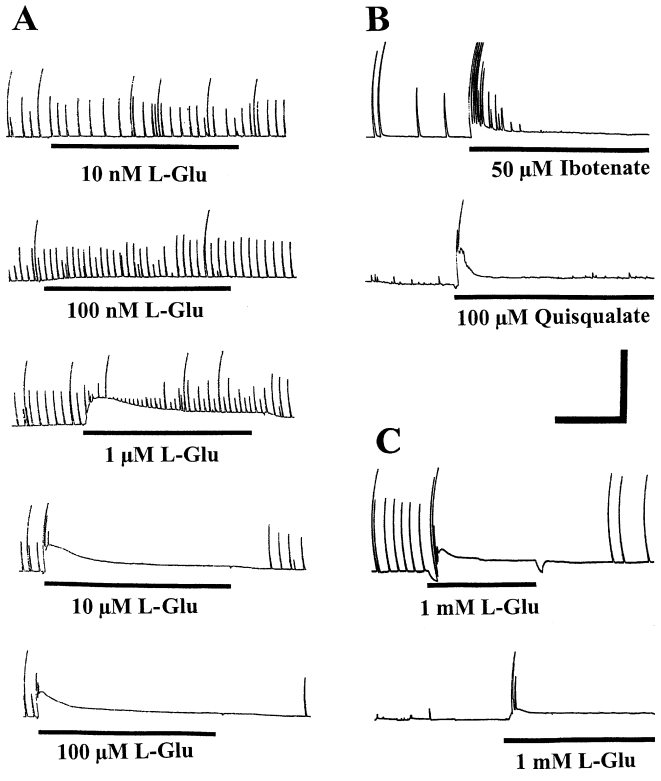

Fig. 1A–C Sample recordings showing the effects of L-glutamate, quisqualate and ibotenate on hindgut contractions. L-Glutamate elicited a slow contraction and suppressed spontaneous hindgut contractions; its effects were dose dependent (**A**). Ibotenate and quisqualate mimicked the effects of L-glutamate (**B**). The effects of L-glutamate might be perceived as inhibitory or excitatory, depending on the level of spontaneous activity in a given experimental preparation (**C**). Scale bars: vertical = 0.25 mN in all case; horizontal = 30 s in **A** and **B**, 1 min in **C**

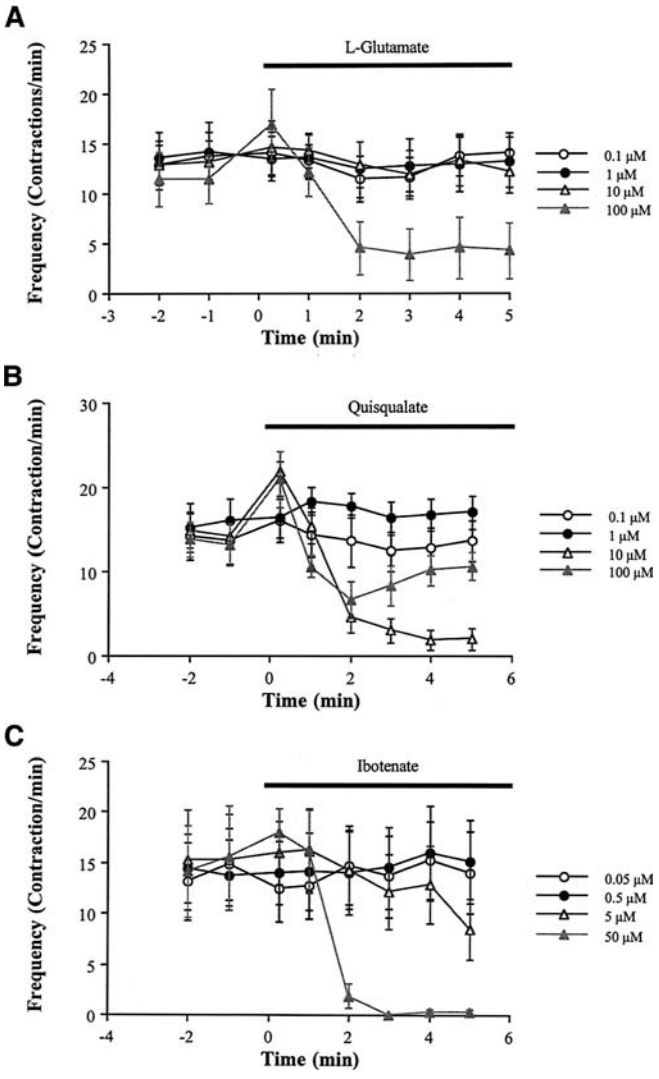

Fig. 2A–C Effects of L-glutamate (**A**), quisqualate (**B**) and ibotenate (**C**) on the frequency of spontaneous contractions of isolated hindguts. Each graph shows the mean frequency of spontaneous hindgut contractions (± SE) as a function of time. Agonists were present in the bathing solution during the period indicated by the *horizontal line*. Number of preparations: 8 for glutamate, 9 for quisqualate, 8 for ibotenate

preparations were more likely to show increases in frequency and amplitude of spontaneous contractions, even though this was only a transient effect. In virtually all preparations, spontaneous contractions were suppressed at high concentrations of all three agonists. Other than this, effects on spontaneous contractions do not appear to be a sensitive indicator of dose dependency.

In contrast to effects on spontaneous contractions, the effects on tonus comprised a fairly robust and reliable indicator of dose dependency (Fig. 4). The IC_{50} for the effect of L-glutamate on tonus was approximately 20 µmol l^{-1}. Saturating doses of quisqualate and ibotenate were not determined. However, comparison of the dose-response curves indicates that quisqualate is approximately ten times more potent than L-glutamate, and that ibotenate is slightly more potent than L-glutamate.

To determine whether glutamate receptors are present on both circular and longitudinal muscles, contractions were recorded from longitudinal strips of hindgut tissue, each containing a single band of longitudinal muscles, or from narrow rings of tissue. As in other experiments (Mercier and Lee 2002), only the longitudinal strips generated spontaneous contractions (1–2 s in duration). The responses of the longitudinal strips to L-glutamate were very similar to those of whole hindgut tisse (Fig. 5A); an abrupt increase in tonus was usually accompanied by a few rapid contractions, after which rapid contractions were suppressed. Rings of tissue, which did not generate "rapid" (1–2 s) contractions, responded only with slow, sustained contractions, lasting up to a few minutes (Fig. 5B). There was a significant increase in tonus in both strips and rings (Fig. 5C), indicating that both longitudinal and circular muscles respond to L-glutamate.

Four other glutamate receptor agonists were tested and had no effect on hindgut contractions (Table 1). These were domoate (a kainate receptor agonist), AMPA (which activates AMPA receptors), NMDA

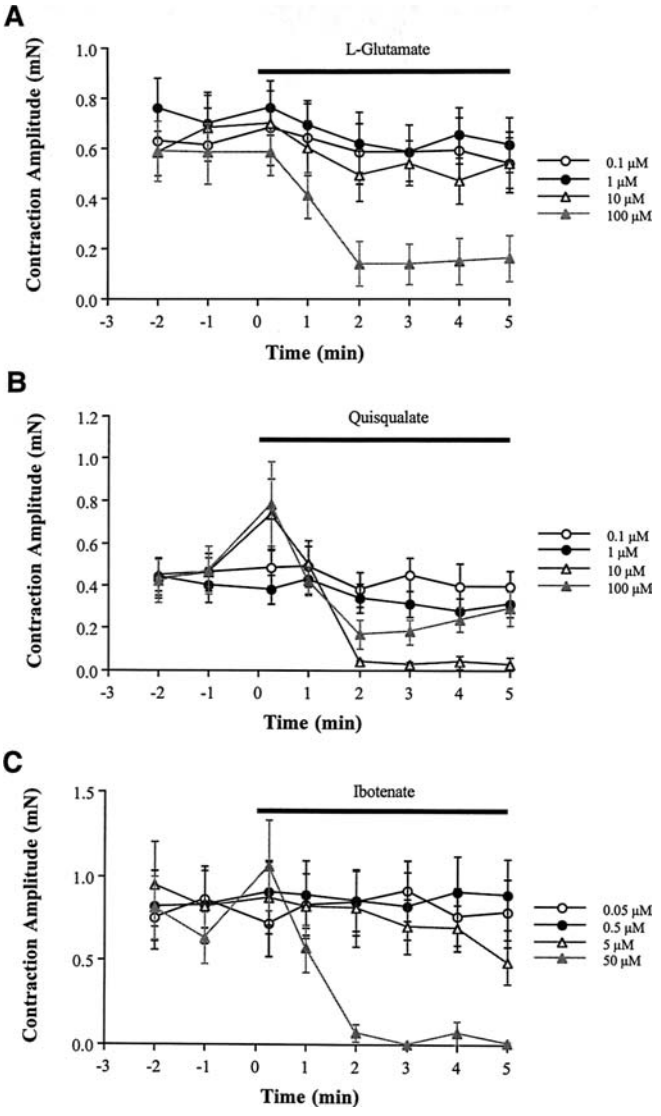

Fig. 3A–C Effects of L-glutamate (**A**), quisqualate (**B**) and ibotenate (**C**) on the amplitude of spontaneous contractions of isolated hindguts. Each graph shows the mean amplitude of spontaneous hindgut contractions (±SE) as a function of time. Agonists were present in the bathing solution during the period indicated by the *horizontal line*. Number of preparations: 8 for glutamate, 9 for quisqualate, 8 for ibotenate

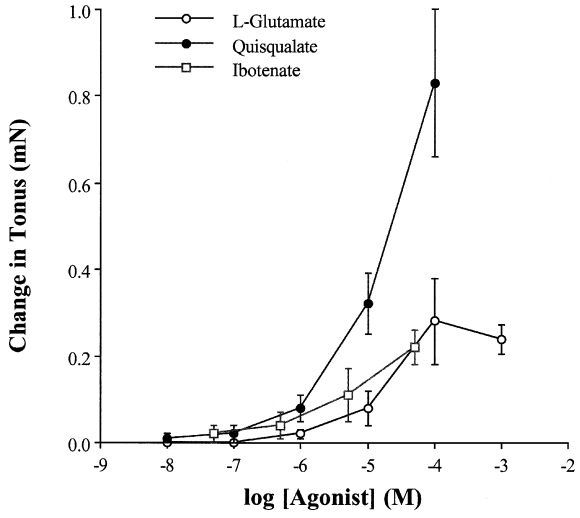

Fig. 4 Effects of L-glutamate, quisqualate and ibotenate on tonus of isolated hindguts. Each point represents the mean (±SE) from 8 preparations (glutamate and ibotenate) or 9 preparations (quisqualate)

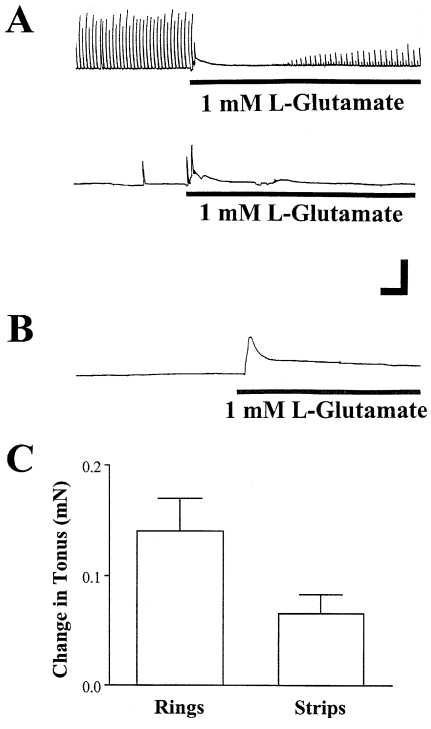

Fig. 5A–C Effects of L-glutamate on circular and longitudinal muscles. Raw recordings show the effects of L-glutamate on longitudinal strips of hindgut tissue (**A**) and on rings of hindgut tissue (**B**). The effects of 1 mmol l^{-1} L-glutamate on tonus in 17 ring preparations and 16 strip preparations are illustrated graphically in **C**. (Scale bars for **A** and **B**: vertical = 0.5 mN; horizontal = 1 min)

(which activates NMDA receptors), and Homo-ACPD (which activates metabotropic glutamate receptors). At the concentrations tested (see Table 1), none of these agents altered the frequency or amplitude of spontaneous contractions, and none had any effect on tonus.

The ability of the glutamate agonists to suppress contractions and the sensitivity of the hindgut to ibotenate both suggest the presence of inhibitory glutamate receptors, which are known to be activated by ibotenate (Cleland 1996; Izawa et al. 1988). We, therefore, examined the effects of picrotoxin, which blocks such receptors. Preparations were treated with 50 µmol l^{-1} picrotoxin for approximately 3 min, after which they were exposed to saline containing 50 µmol l^{-1} picrotoxin and 50 µmol l^{-1} ibotenate for 3 min; the effects of treatment with ibotenate + picrotoxin were compared to the effects of exposure to ibotenate alone. There was no significant difference ($P > 0.1$, Mann-Whitney U-test) between contraction frequencies for preparations

Table 1 Summary of experiments with glutamate receptor agonists (*AMPA* (±)-α-amino-3-hydroxy-5-methyl-isoxazole-4-propionoic acid; *NMDA N*-methyl-D-aspartate; *Homo-APCD* (1S,3R)-1-amino-1-carboxy-cyclopentane-3-acetic acid)

Compound	Concentrations tested	Preparations	Tonus	Spontaneous contractions
L-Glutamate	10 nmol l^{-1}–1 mmol l^{-1}	8	Increase	Decrease
Quisqualate	10 nmol l^{-1}–100 μmol l^{-1}	9	Increase	Decrease
Ibotenate	50 nmol l^{-1}–50 μmol l^{-1}	8	Increase	Decrease
Domoate	10 nmol l^{-1}–10 μmol l^{-1}	6	No effect	No effect
AMPA	10 nmol l^{-1}–10 μmol l^{-1}	6	No effect	No effect
NMDA	10 nmol l^{-1}–100 μmol l^{-1}	6	No effect	No effect
Homo-APCD	250 nmol l^{-1}–250 μmol l^{-1}	6	No effect	No effect

exposed to 50 μmol l^{-1} ibotenate (7.8 ± 3.3 contractions min^{-1}; $n = 6$) or to 50 μmol l^{-1} ibotenate + 50 μmol l^{-1} picrotoxin (10.2 ± 3.6 contractions min^{-1}; $n = 6$ preparations). Not surprisingly, the increase in tonus caused by ibotenate (0.24 ± 0.13 mN; $n = 6$ preparations) was not significantly altered by applying ibotenate in the presence of 50 μmol l^{-1} picrotoxin (0.37 ± 0.13 mN; $n = 6$ preparations; $P > 0.1$, Mann-Whitney *U*-test). Thus, 50 μmol l^{-1} picrotoxin failed to antagonize the effects the effects of 50 μmol l^{-1} ibotenate.

We also examined the effects of JSTX-3, which irreversibly blocks the quisqualate-type glutamate receptors associated with arthropod skeletal muscles (Kawai et al. 1991; Miwa et al. 1987). One group of preparations was treated with 1 mmol l^{-1} L-glutamate, and a second group was treated with 1 mmol l^{-1} L-glutamate after a 5-min exposure to 100 μmol l^{-1} JSTX-3. In the absence of JSTX-3, L-glutamate completely suppressed spontaneous contractions in all preparations ($n = 6$) after 2 min (Fig. 6A). Following pre-treatment with JSTX-3, 1 mmol l^{-1} L-glutamate suppressed contractions in four of six preparations. Although the results suggest partial antagonism (Fig. 6A), there was no significant difference between control and JSTX-treated groups ($P > 0.1$ at 2 min; $P > 0.05$ at 3 min; Mann-Whitney *U*-test). However, the rise in tonus elicited by L-glutamate was significantly lower following exposure to JSTX-3 (Fig. 6B; $P < 0.001$, Mann-Whitney *U*-test). Thus, the effect of L-glutamate on tonus, at least, is antagonized by JSTX-3. Interestingly, the frequency of spontaneous phasic contractions was significantly higher in saline after exposure to JSTX-3 than in preparations not treated with JSTX-3 ($t = -1$ min in Fig. 6; Mann-Whitney *U*-test, $P < 0.025$).

The suppression of spontaneous hindgut contractions at high agonist concentrations might be caused by receptor desensitization or by a strong depolarization. The latter effect could release large amounts of Ca^{2+} from intracellular stores, which would eventually produce a single, long-lasting contraction and render the cell incapable of responding to other stimuli until the Ca^{2+} is re-sequestered. We examined the influence of depolarization by applying saline with elevated K^+ concentrations. Increasing extracellular K+ concentration (from 5.3 mmol l^{-1} to 30 mmol l^{-1}) mimicked all the effects of L-glutamate, quisqualate and ibotenate (Figs. 7 and 8). There was an increase in tonus, a transient increase in the frequency of spontaneous contractions, and a subsequent reduction or complete suppression of spontaneous contractions.

Discussion

The present data indicate that the crayfish hindgut contains glutamate receptors which induce contracture in both circular and longitudinal muscles. The glutamate

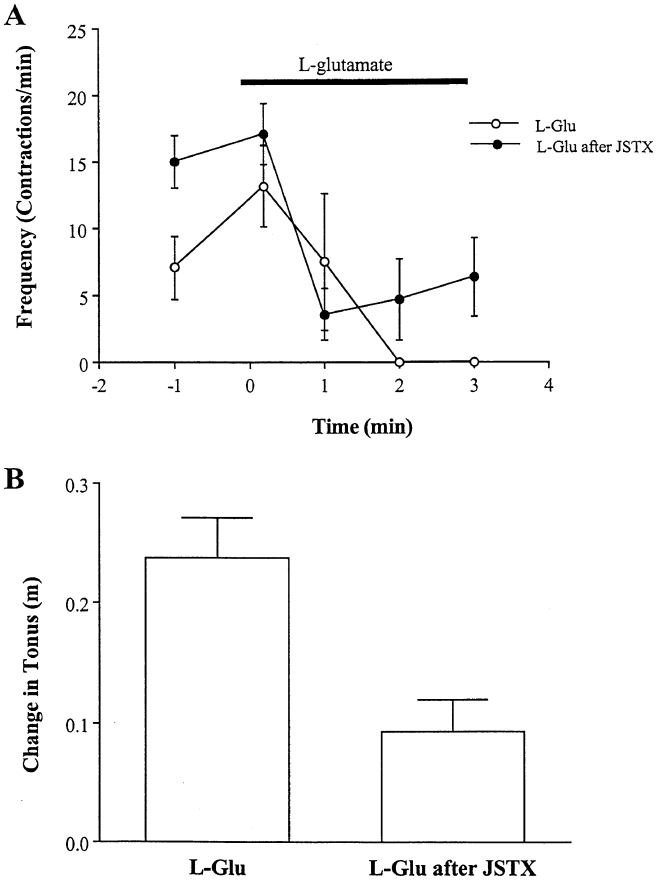

Fig. 6A,B Effects of glutamate with or without pre-treatment with Joro spider toxin (JSTX-3). Changes in contraction frequency (**A**) were recorded in preparations showing spontaneous contractions ($n = 6$ for glutamate alone and $n = 6$ for glutamate following exposure to 100 μmol l^{-1} JSTX-3). L-Glutamate (at 1 mmol l^{-1}) was applied during the period shown by the horizontal bar (**A**). Changes in tonus (**B**) were recorded from all preparations, regardless of whether they showed spontaneous contractions ($n = 8$ for each group)

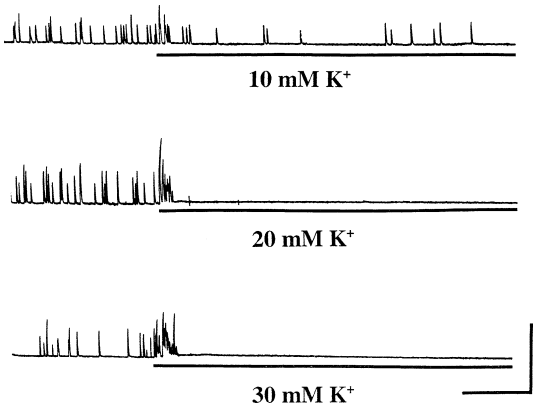

Fig. 7 Sample recordings showing effects of elevated extracellular K^+ concentration on contractions of isolated crayfish hindguts. Crayfish saline containing elevated K^+ (at concentrations indicated in *parentheses*) was applied during the period shown by the *horizontal bar*. Calibration bars: vertical = 0.7 mN; horizontal = 1 min

receptors appear to be primarily of the quisqualate-type, since quisqualate was the most potent of the agonists examined, but they also show some sensitivity to ibotenate. They are not activated by NMDA, AMPA, or an activator of metabotropic glutamate receptors, and they are antagonized (at least partially) by JSTX-3. These receptors, therefore, appear to be pharmacologically very similar (if not identical) to glutamate receptors associated with arthropod skeletal muscles (Takeuchi and Takeuchi 1964; Usherwood 1967; Usherwood et al. 1968; Atwood 1982; Kawai et al. 1991; Miwa et al. 1987; Usherwood and Blagbrough 1992).

The effects of L-glutamate were mimicked by quisqualate and ibotenate, two agonists of glutamate receptors. However, three other ionotropic glutamate receptor agonists (NMDA, domoate, and AMPA) and a metabotropic glutamate receptor agonist (Homo-ACPD) did not elicit any effects on the hindgut preparation. A major distinction between invertebrate and vertebrate quisqualate-activated ionotropic receptors is the insensitivity of the former to AMPA-mediated activation (Usherwood and Blagbrough 1992). The evidence presented in this study, specifically the sensitivity to quisqualate but not AMPA, suggests that the receptors under investigation may be homologous to the quisqualate receptors extensively characterized in the locust leg muscle (Gration et al. 1979; Gration and Usherwood 1980; Usherwood 1981). Ibotenate is a non-selective agonist that can activate NMDA receptors (Marret et al. 1996; Inglis and Semba 1997), metabotropic glutamate receptors (Palucha et al. 2000; Scholz 1994) and inhibitory glutamate receptors (Cull-Candy and Usherwood 1973; Izawa et al. 1988).

Three distinct effects on hindgut contractions were observed: an increase in tonus; a brief, transient increase in contraction frequency; and suppression of spontaneous phasic contractions. The suppression of phasic contractions that we observed in response to 100 μmol l^{-1} L-glutamate probably explains why Florey (1961) initially

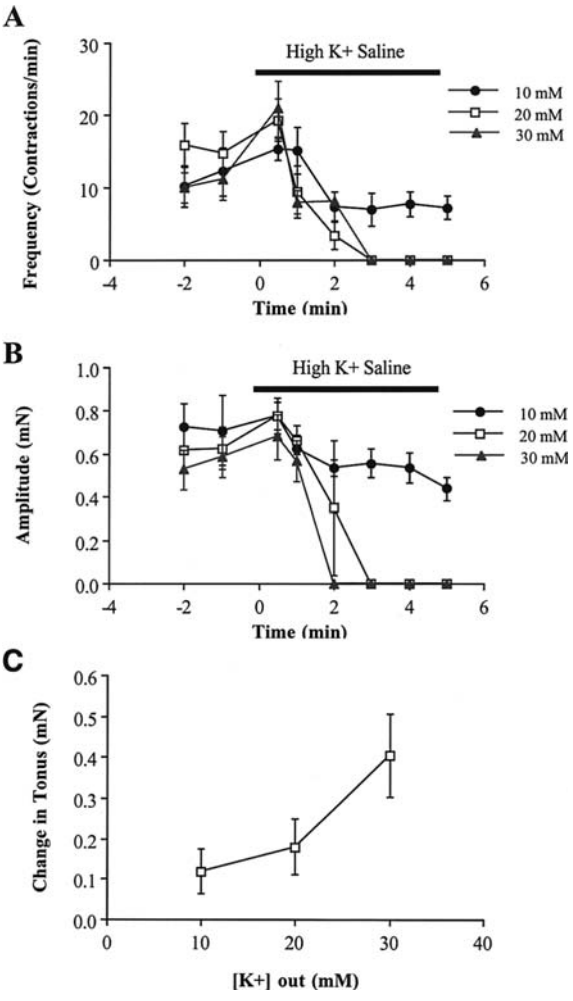

Fig. 8A–C Elevated extracellular K^+ concentration mimics the effects of L-glutamate on hindgut contractions. Frequency of contractions (**A**) and contraction amplitude (**B**) are plotted as functions of time. Crayfish saline containing elevated K^+ (at concentrations indicated in *parentheses*) was applied during the period shown by the *horizontal bar*. Changes in tonus (**C**) are plotted against K^+ concentration. Number of preparations = 5 in all cases

concluded that glutamate is inhibitory. The other effects of L-glutamate (the increase in the tonus and transient increase in contraction frequency) support the conclusion of Jones (1962) that glutamate is excitatory. The interpretation of whether glutamate's effects are excitatory or inhibitory can be influenced by the degree of spontaneous activity in preparations (e.g., Figs. 1C and 5A). Although we did not examine more than one species, our results suggest that the original conclusions of Florey (1961) and Jones (1962) reflect differences in interpretation of the data rather than species differences. In fact, our results demonstrate excitatory actions of glutamate in *P. clarkii*, for which Florey concluded the actions of glutamate to be inhibitory.

Glutamate can elicit inhibitory actions by activating inhibitory glutamate receptors (IGluRs) which open Cl^- channels, and ibotenate is a potent agonist of these receptors (Cleland 1996). Such inhibitory actions occur

in insect hindgut, where ibotenate suppresses spontaneous contractions (Izawa et al. 1988). Ibotenate did not appear to increase tonus or elicit a transient increase in frequency of spontaneous contractions in insect hindgut (Izawa et al. 1988). In crayfish hindgut, however, suppression of spontaneous contractions by ibotenate often followed a transient increase in frequency (e.g., Fig. 1B), and ibotenate significantly increased tonus. Thus, although ibotenate may preferentially activate IGluRs in insect hindgut, it appears to activate excitatory glutamate receptors in crayfish hindgut. We examined the possibility that ibotenate activates IGluRs in addition to its excitatory effects by testing picrotoxin, an IGluR antagonist (Cleland 1996). At 50 μmol l^{-1}, picrotoxin failed to antagonize ibotenate-induced suppression of hindgut contractions; this is five times the concentration needed to block the effect of 50 μmol l^{-1} ibotenate on insect hindgut (Izawa et al. 1988). Moreover, spontaneous contractions of crayfish hindguts were suppressed by quisqualate at concentrations as low as 10 μmol l^{-1} (Fig. 2), indicating that the suppression of contractions is not selectively induced by ibotenate. Thus, our data do not support the notion that IGluRs are activated by ibotenate in crayfish hindgut.

One factor that might contribute to the suppression of spontaneous phasic contractions is receptor desensitization. The pattern of transient excitation followed by prolonged suppression of contractions that we observed is remarkably similar to the effects of high concentration of glutamate on the retractor unguis muscle of the locust, reported by Usherwood and Machili (1966). In that study, depolarization was elicited by iontophoretic application of small quantities of glutamate. Perfusion of the bath with glutamate caused a large initial depolarization, followed by suppression of the effects of iontophoretically applied glutamate. If desensitization were to account for suppression of phasic contractions in the crayfish hindgut, the spontaneous contractions would presumably be evoked by spontaneous release of glutamate from nerve terminals within the hindgut plexus. There is no evidence that the hindgut plexus exhibits any spontaneous electrical activity in crustaceans, and it seems unlikely that spontaneous release of small numbers of quanta of transmitter from synaptic terminals would be sufficient to generate even weak contractions. The hindgut plexus is thought to function as the site for release of transmitters in response to impulses in motor neurons whose cell bodies lie within the central nervous system (Wales 1983; Winlow and Laverack 1972b, 1972c). Although the spontaneous hindgut contractions are thought to be myogenic (Winlow and Laverack 1972a), no one has definitively ruled out the possibility that they result from spontaneous release of glutamate from nerve terminals or that the hindgut nerve plexus is spontaneously active. If phasic contractions were caused by spontaneous release of L-glutamate, one would expect an appropriate glutamate antagonist to reduce such spontaneous contractions, decreasing their amplitude and, possibly, their frequency. However, the frequency of spontaneous phasic contractions was actually higher in JSTX-3, which reduces L-glutamate-induced contractures. Thus, our data do not support the notion that phasic contractions result from spontaneous release of glutamate. Nonetheless, we cannot rule out the possibility that such contractions might involve JSTX-insensitive glutamate receptors or that they might result from the release of transmitters other than L-glutamate.

Another factor that probably contributes to the suppression of spontaneous contractions is strong depolarization, particularly when glutamate agonists are applied at high concentrations. The results of the experiments with high [K^+] saline support this idea, since this treatment mimicked all the effects of glutamate, quisqualate and ibotenate. In each trial in which suppression of rapid contractions occurred, during the onset of agonist application there was either a strong contraction or a burst of contractions followed by a partial or complete lack of phasic contractions. This could be the result of a high influx of calcium, or calcium overloading, in the longitudinal muscle cells responsible for the phasic contractions.

Overall, the pharmacological properties of glutamate receptors associated with crayfish hindgut muscles appear to be similar to those of arthropod skeletal muscles. The receptors are of the quisqualate type, they are antagonized (at least partially) by JSTX-3, they mediate excitatory effects, and they may show desensitization. While these experiments shed some light onto the pharmacology of the receptors mediating contractions in the hindgut, questions remain concerning the origin of the rhythmic contractions and the involvement of the putative quisqualate-type GluRs in regulating the physiological behavior of the hindgut.

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