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A STUDY OF THE INTERACTIONS BETWEEN GLUTAMATE AND ASPARTATE AT THE LOBSTER NEUROMUSCULAR JUNCTION

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1 The depolarizations produced by bath-applied or iontophoretically applied glutamate and aspartate were recorded from lobster muscle fibres by means of intracellular microelectrodes.

2 Bath-applied glutamate or aspartate evoked reversible, membrane depolarizations; however, responses to repeated applications of aspartate decreased progressively in amplitude until a plateau level was attained. Repeated applications of glutamate, kainate, domoate or quisqualate did not produce a similar effect.

3 After a dose of glutamate, responses to bath-applied aspartate were enhanced. Responses to other depolarizing agonists were little affected by previous administration of glutamate. Aspartate dose-depolarization curves were therefore constructed after initial aspartate responses had stabilized. The log–log transforms of the aspartate and glutamate curves had limiting slopes of 0.8 and 2.1 respectively.

4 Iontophoretic application of aspartate to single glutamate-sensitive sites produced small depolarizations with slow time course, compared with the glutamate potentials. When aspartate and glutamate were pulsed simultaneously from a twin-barrelled pipette, the resultant glutamate potential was enhanced. It is suggested that this potentiation was due to summation of agonist concentrations in the receptor region interacting with a second-order dose-response relationship.

5 Bath-applied aspartate increased the amplitude and prolonged the half-decay time of the glutamate potential. This effect was particularly noticeable when the glutamate potential was of slow time course.

6 It is proposed that bath-applied aspartate has an agonist effect whose magnitude is possibly exaggerated by concomitant release of glutamate and/or inhibition by glutamate of aspartate uptake. This agonist action of aspartate is thought to be exerted mainly on extrajunctional areas of the glutamate-sensitive sites.

Introduction

The neuronal depolarizing action of L-glutamate and L-aspartate has been compared at various sites in the mammalian central nervous system (Curtis, Duggan, Felix, Johnston, Tebēcis & Watkins, 1972; Duggan, 1974; Biscoe, Headley, Lodge, Martin & Watkins, 1976). Glutamate is also believed to be the excitatory neurotransmitter at the crustacean neuromuscular junction (Kravitz, Slater, Takahashi, Bownds & Grossfeld, 1970; Gerschenfeld, 1973) and is present (together with aspartate) in relatively large concentrations in excitatory axons of the lobster and crab (Evans, 1973; Shank, Freeman, McBride & Aprison, 1975). In Crustacea, aspartate has been proposed as a co-transmitter, being released together with glutamate from the excitatory nerve terminals (Kerkut & Wheal, 1974; Freeman, 1976).

It was originally noted by Kravitz *et al.* (1970) that small concentrations of bath-applied aspartate greatly enhanced the depolarizing action of glutamate on lobster muscle when both amino acids were applied in combination (see also Kerkut & Wheal, 1974; Shank & Freeman, 1975; Crawford & McBurney, 1977a). A similar phenomenon was also demonstrated with iontophoretically-applied glutamate and aspartate (Shank, Wang & Freeman, 1977). According to Shank *et al.* (1975) and Shank & Freeman (1975) this enhancement was due to a facilitation of the glutamate/receptor interaction. However, Crawford & McBurney (1977a, b) have suggested that on crab muscle, aspartate might potentiate glutamate action by blocking glutamate uptake. On crayfish muscle,

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Dudel (1977) concluded that such a potentiation resulted from a suppression of glutamate desensitization by aspartate. During the course of our study of the depolarizing actions of aspartate and glutamate on lobster muscle we noted a new interaction phenomenon; namely, that the effect of bath-applied but not of iontophoretically-applied aspartate was greatly influenced by a previous 'conditioning' application of glutamate. We suggest that this effect, if generally occurring, could certainly influence any interpretations of glutamate/aspartate 'synergism' and also of aspartate dose-response data obtained on other crustacean muscle preparations.

Methods

Experiments were conducted at room temperature (20–24°C) on the claw-opener muscle of the walking leg of the lobster *Homarus vulgaris* which was exposed and prepared for intracellular recording as previously described (Constanti & Nistri, 1976a). The membrane potential of single superficial fibres was recorded with respect to earth by means of a microelectrode (filled with 1.5 M potassium citrate) inserted at the centre of the fibre. A second microelectrode (filled with 0.6 M potassium sulphate) was inserted within 50 μm of the voltage electrode in order to pass hyperpolarizing current pulses (0.25 Hz; 800 ms) through the membrane. The resultant electrotonic potentials were displayed on a storage oscilloscope and recorded on a chart recorder. The opener muscle was continuously superfused *in situ* with lobster saline solution of the following composition (mM): NaCl 522, KCl 12, $CaCl_2$ 21, $MgCl_2.6H_2O$ 5 and Tris maleate 10, adjusted to pH 7.6 with 0.1 N NaOH. All drugs were dissolved in this solution and adjusted to pH 7.6 before use.

Under these conditions, the current/voltage relation of the lobster opener fibre membrane was essentially linear (for membrane potential changes $\leqslant \pm 20$ mV); the dose-response relations to glutamate and aspartate could therefore be compared without the necessity of including γ-aminobutyric acid (GABA) in the bathing medium in order to linearize the current/voltage relation (see Shank & Freeman, 1975).

Iontophoretic glutamate potentials were obtained by the method of Takeuchi & Takeuchi (1964), in which negative current pulses were applied through a microelectrode (30-50 MΩ) filled with 1 M L-Na glutamate (adjusted to pH 8 with NaOH) positioned over a glutamate-sensitive area of the muscle fibre surface (retaining currents = +10 to +50 nA). The potentials were recorded by an intracellular electrode placed about 200 μm away from the sensitive spot and photographed from the oscilloscope screen with a Polaroid camera. As the average space constant of a resting lobster fibre is about 2 mm (on the basis of 'short' cable theory; see Constanti, 1977) the estimated % attenuation of a glutamate potential recorded in this way was 7%.

Double-barrelled microelectrodes (50-70 MΩ) were also prepared. One barrel was usually filled with 1 M L-Na glutamate (pH 8) and the second with 0.5 to 1 M L-aspartate (pH 8 to 9). In order to check for possible barrel interaction, one barrel was sometimes filled with NaCl (1 M, pH 8); large currents (up to ±1 μA) through the saline barrel of a glutamate/saline twin pipette produced no detectable effect; moreover, the glutamate potential was unaffected by simultaneous pulses through the saline barrel. However, a slight reduction of the glutamate response was sometimes seen when high (>50 nA) retaining currents were applied to the saline pipette.

Drugs

L-Glutamate (sodium salt) and kainic acid were purchased from Sigma and L-aspartic acid from Hopkin and Williams. Domoic and quisqualic acids were gifts from Professor T. Takemoto (Tohoku University, Sendai, Japan). All other compounds were of reagent grade. Aspartate samples were checked for contamination with glutamate by paper chromatography (butanol/acetic acid/water: 4/1/1).

Results

Responses to bath-applied glutamate and aspartate

Our aim was to compare glutamate and aspartate-evoked depolarizations on lobster muscle, undistorted by the presence of GABA in the bathing medium (see Methods and Shank & Freeman, 1975). Bath-applied glutamate (50 to 400 μM) produced reproducible membrane depolarizations characterized by a relatively rapid rate of onset and offset and by 'fading' during exposure to high concentrations (see Constanti & Nistri, 1976b). Aspartate (1 to 10 mM) also reversibly depolarized the muscle fibres although compared with glutamate, the rate of onset and offset of an aspartate response was usually slower (Shank & Freeman, 1975). Moreover, we noted that the depolarizations produced by aspartate (1 mM), applied at regular dose intervals of 8 to 10 min at the start of an experiment (when no other drugs had been applied) decreased progressively in amplitude until a 'plateau' level was attained (Figure 1). At this point, the resting membrane input resistance was the same as the control and the fibres still responded to glutamate (50 or 100 μM) in a characteristic *reproducible* manner. Application of aspartate 2 to 8 min after the end of a test dose of glutamate now evoked a response consistently larger than that obtained just before the ad-

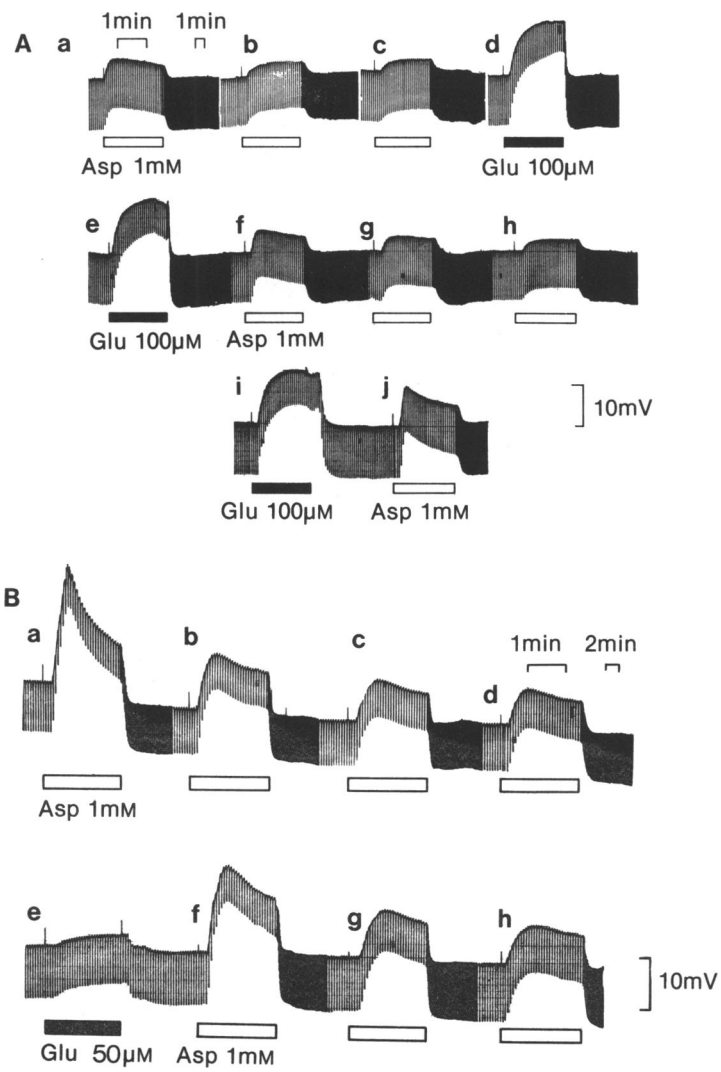

Figure 1 Depolarizations evoked by L-aspartate (1 mM, open bars) and L-glutamate (50 μM or 100 μM, filled bars) recorded at the centre of single lobster muscle fibres. Downward deflections represent hyperpolarizing electrotonic potentials evoked by intracellular current pulses (800 ms; 100 nA in (A); 150 nA in (B), different preparations). (A): (a to j), continuous record reading left to right. (a to c), successive responses to aspartate (dose interval 8 min) obtained at beginning of an experiment; note progressive reduction in response amplitude, not seen with glutamate (Glu) (100 μM, d and e). (f), Enhancement of response to aspartate when applied 6 min after glutamate response (e) (compare with (a)). (g to h), Further progressive reduction in aspartate response until (j) when aspartate (Asp) was applied 2 min after 100 μM glutamate (i); note the greater increase in amplitude relative to (f). Note also the fading effects of aspartate in (a), (f) and (j), not seen with glutamate. Resting potential of fibre = − 80 mV. (B): (a to h), continuous record. Similar sequence to (A); however, in this fibre note the large and rapidly fading depolarization produced by the first application of aspartate (a). Subsequent responses to aspartate showed progressive reduction in amplitude (dose interval = 10 min). (f), Enhancement of aspartate response applied 2 min after 50 μM glutamate (e) followed by progressive reduction in amplitude (g to h). Resting potential = −76 mV. Chart-speed was slower during decline of some responses.

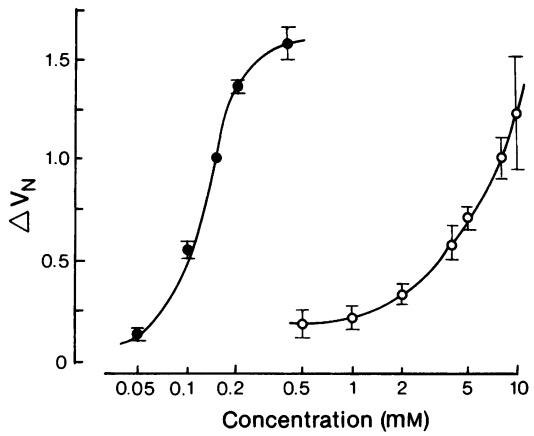

Figure 2 Normalized log dose-depolarization curves for glutamate (●) and aspartate (○) obtained on single fibres. Each aspartate curve was constructed after initial responses to aspartate (1 mM) had stabilized (see text). Ordinate scale: membrane depolarization (normalized with respect to 150 μM glutamate response on same fibre). Abscissa scale: concentration of amino acid added to bathing solution. Points represent mean of between 3 to 6 determinations on different preparations. Vertical lines show s.e. mean.

ministration of glutamate; subsequent aspartate responses then decreased progressively to a stable level as previously observed (Figure 1).

It might be argued that insufficient time was allowed between successive aspartate applications to allow for full recovery of sensitivity. However, even after a 30 min wash period, aspartate responses continued to decrease in amplitude until glutamate was reapplied; the subsequent aspartate response was then enhanced. This progressive decrease in aspartate responses and the influence of a 'conditioning' dose of glutamate on this decline was present in all fibres studied. Therefore, in order to construct meaningful aspartate dose-depolarization curves, aspartate (1 mM) was applied repeatedly (with intermediate washing) until responses stabilized; higher doses (up to 10 mM) were then applied to construct the aspartate curve (with repetition of lower or higher doses to check for reproducibility); finally, the glutamate curve was constructed. Both glutamate and aspartate depolarizations were normalized with respect to the 150 μM glutatamate response and expressed as ΔV_N (Figure 2). Definite maxima for either curve could not be attained because of desensitization and the risk of fibre contraction. The log–log transformations of each curve (not shown) had limiting slopes of 2.1 and 0.8 for glutamate and aspartate respectively (see Constanti & Nistri, 1976b) possibly indicating that more than one molecule of glutamate, but perhaps only a single molecule of aspartate was interacting with a single receptor site. This finding does not however imply that glutamate and aspartate were acting on common receptors.

It was important to test whether other depolarizing agonists could behave like aspartate on this preparation. Thus, when glutamate (50 μM) was used as test agonist, successive responses were reproducible and unaffected by a previous larger dose of glutamate (100 μM; Figure 3a to d); this suggests that the enhancement of aspartate responses was not merely due to small amounts of glutamate remaining in the region of the receptors. Moreover, 3 min after the end of an aspartate response (1 mM) a response to 50 μM glutamate was usually slightly depressed relative to control (Figure 3e to g). Figure 4 shows that similar results were obtained when 2 μM quisqualic acid, 50 μM domoic acid or 1 mM kainic acid were used as test agonists (Shinozaki & Shibuya, 1974a, b; 1976; Constanti & Nistri, 1976b). The effects seen with bath-applied aspartate therefore seemed to be a peculiar feature of the action of this amino acid on the lobster muscle membrane. We subsequently investigated whether a similar interaction between glutamate and aspartate could occur when these agents were applied iontophoretically to single sensitive sites.

Responses to iontophoretically-applied glutamate and aspartate

Typical glutamate potentials evoked by increasing iontophoretic currents are shown in Figure 5a. The progressive increase in the time-to-peak (T_{max}) of these potentials (measured from the onset of the current pulse) was probably due to some desensitization or saturation of receptors immediately under the pipette tip and contributions from more distant receptors (Del Castillo & Katz, 1955). Figure 5b shows the relationship between the applied charge of glutamate and the resulting depolarization; the log–log transformation of this curve (not shown) gave a limiting slope of 2.6 (the mean (±s.d.) slope from 4 experiments was 2.1 ± 0.46) indicating that the iontophoretic response was dependent upon the second power of the glutamate concentration (cf. Dudel, 1975). We assumed a direct relation between glutamate release and iontophoretic currents applied, since we found that the 'dose'-response curve obtained as above resembled that obtained with the 'increment' method (Dudel, 1975) in which a twin glutamate/glutamate pipette was used to apply equieffective doses of glutamate alternately and then simultaneously to the same spot. This avoided the use of iontophoretic current as a measure of applied glutamate.

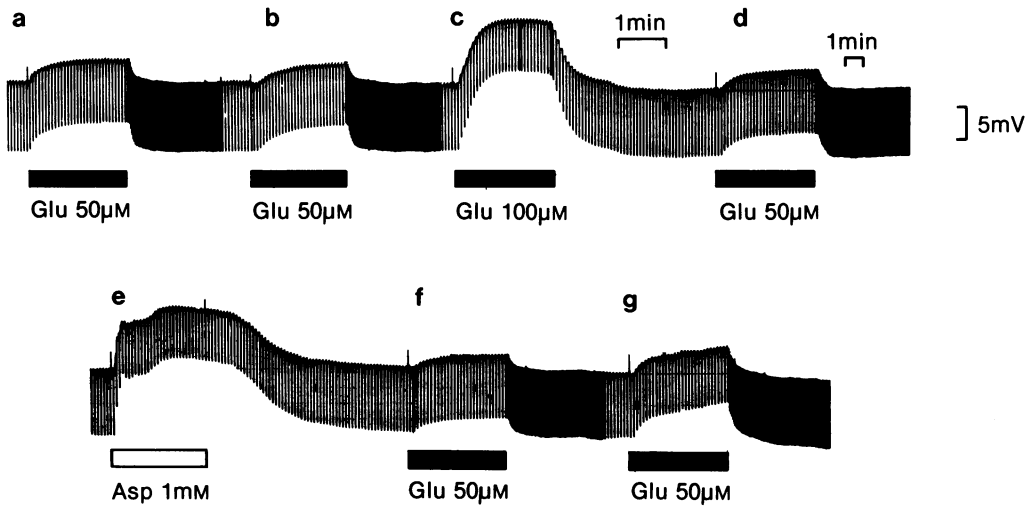

Figure 3 Depolarization evoked by glutamate (50 μM and 100 μM, filled bars) and aspartate (1 mM, open bar) in a single muscle fibre (resting potential = − 74 mV; current pulses = 200 nA). (a to g), Continuous record; (a and b), control glutamate responses (50 μM, dose-interval = 8 min). Note good reproducibility. (d) Glutamate (50 μM) response obtained 3 min after larger glutamate dose (100 μM, c); (f) glutamate (50 μM), response 3 min after aspartate (1 mM); (e) is slightly depressed but subsequently returns to control level (g).

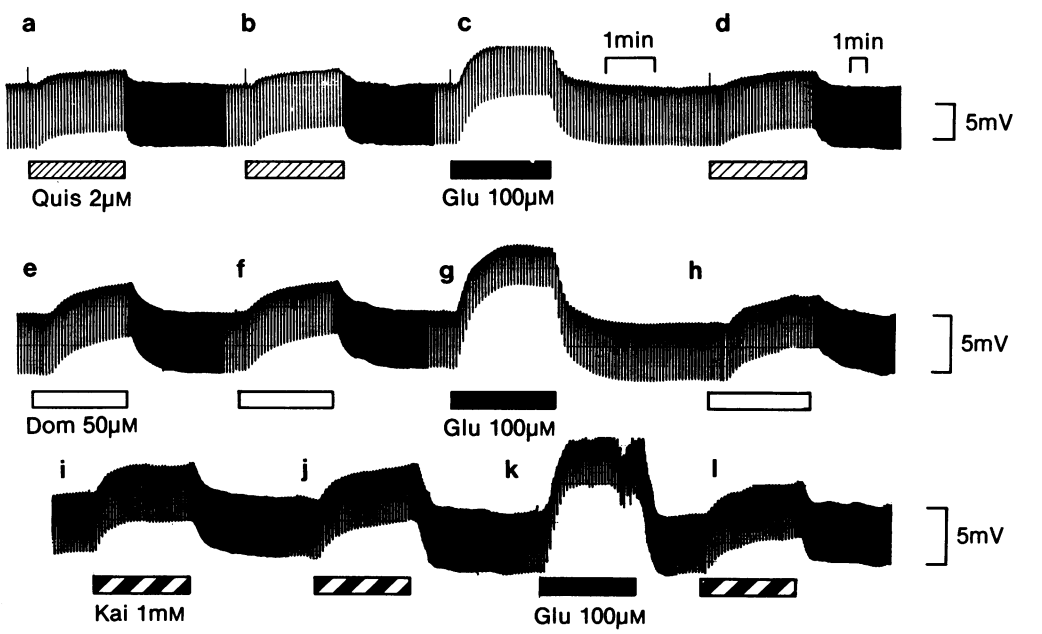

Figure 4 Depolarizations evoked by glutamate (Glu 100 μM, filled bars), quisqualic acid (Quis 2 μM, hatched bars), domoic acid (Dom 50 μM, open bars) and kainic acid (Kai 1 mM, thick hatched bars) in single lobster fibres (see legend, Figure 1). Sequence (a to d) is similar to that of Figure 3 (a to d), and shows that quisqualate (2 μM) responses were consistent and unaffected by a previous application of glutamate (100 μM) (resting potential of fibre = −74 mV; current pulses = 200 nA); (e to h) and (i to l) are similar ssquences carried out in a different fibre with domoic acid (50 μM) and kainic acid (1 mM) as test agonists respectively. Note that domoate and kainate responses were slightly depressed after glutamate (100 μM) (resting potential = −80 mV; current = 120 nA).

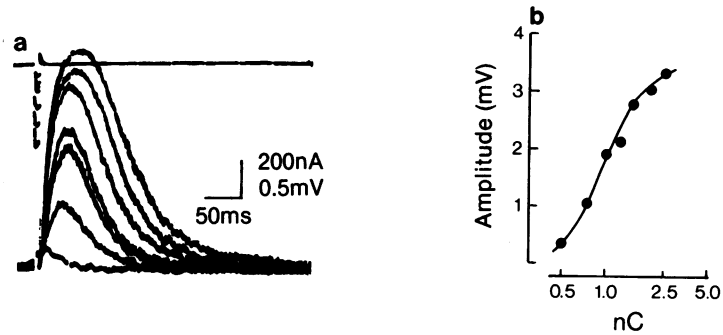

Figure 5 (a) Superimposed membrane depolarizations (lower beam) evoked at a single sensitive site by current pulses of increasing intensity (upper beam) applied through an iontophoretic glutamate pipette (traces read successively from below and were recorded at 10 s intervals). Retaining current = +20 nA; resting potential = −74 mV. Note that larger currents produce potentials with longer time-to-peak. (b) Log 'dose'-depolarization curve plotted from data in (a). Ordinate scale: amplitude (mV) of iontophoretic glutamate potential; abscissa scale: Coulomb strength of ejection current (retaining current subtracted). The fibre sensitivity at the approximate mid-point of this curve was 1.9 mV/nC.

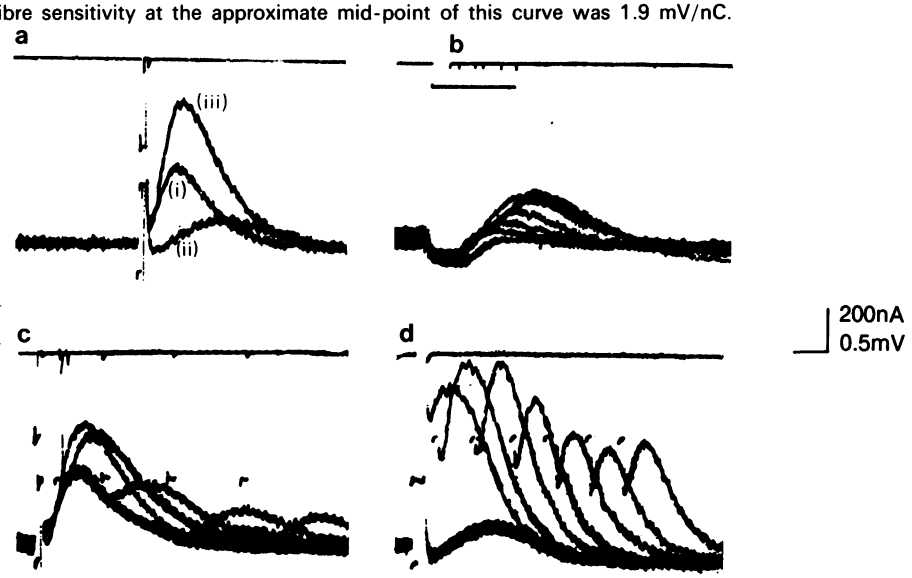

Figure 6 (a) Comparison of depolarizations produced by iontophoretically-applied glutamate and aspartate at same spot on opener muscle membrane; the drugs were ejected from separate barrels of a twin pipette. Three sweeps were superimposed at 30 s intervals; (i), control glutamate potential, (ii) aspartate potential, note slower time course, (iii) potential produced when glutamate and aspartate pulses were applied simultaneously. Retaining currents on both barrels = +10 nA. (b) Superimposed aspartate potentials (6 sweeps). The iontophoretic current pulse amplitude was kept constant (120 nA) and the pulse width increased successively from 75 to 440 ms (retaining current on glutamate barrel maintained throughout). (c) Interaction between glutamate and aspartate potentials (5 sweeps superimposed). In each sweep, one glutamate and one aspartate potential were obtained in that order. The interval between pulses was decreased progressively from 550 ms to 0 ms (coincident). Note greater amplitude of coincident potential (relative to control glutamate) and progressively increasing amplitude of aspartate potential. (d) Interaction between aspartate and glutamate potentials (7 sweeps superimposed, one aspartate and one glutamate potential obtained per sweep, in that order). Interval between aspartate and glutamate was initially 550 ms, and was decreased by 100 ms steps to 0 ms (coincident). Note progressive increase in amplitude of glutamate response. All potential amplitudes were recorded from peak to base line. In (c) and (d) where one potential occurred during time course of another, the amplitude was measured from peak to extrapolated falling phase of preceding potential. In all records, upper beam represents iontophoretic current (calibration = 200 nA). For (a), (c) and (d), voltage/time calibration = 0.5 mV, 100 ms; For (b) it was 0.5 mV, 200 ms. All records were obtained at same membrane site (resting potential of fibre = −76 mV).

A twin iontophoretic pipette was also used to apply glutamate or aspartate to the membrane. After locating a glutamate-sensitive site, a negative current pulse applied to the aspartate barrel gave a small depolarization with a slow time course. When glutamate and aspartate were pulsed simultaneously, the resultant glutamate potential was enhanced by 90% and its time course slowed (Figure 6a). It was usually difficult to obtain a clear aspartate 'dose'-depolarization curve by increasing the iontophoretic current. However, when the pulse width was increased (current amplitude constant) progressively larger and slower potentials were obtained (Figure 6b). The graded nature of these potentials suggests a postsynaptic membrane action rather than a release of transmitter from excitatory terminals because in the latter case discrete unitary potentials (i.e. m.e.j.ps) might be expected (Takeuchi & Takeuchi, 1964; Dowson & Usherwood, 1972). They were also unlikely to be current artefacts or due to glutamate release from the adjacent barrel since no potential was seen when the polarity of the aspartate current was reversed, and the time course of the aspartate potential was consistently slower than that of the glutamate potential; this difference in time course was probably not due to tip separation since a twin pipette with glutamate in both barrels gave potentials of similar time course and amplitude, irrespective of the barrel used.

In 5 out of 8 experiments where sufficiently large aspartate potentials were obtained, repeated applications (pulse rate up to 4 Hz) did not produce a decline in potential amplitude (cf. bath-application experiments). On other occasions, no aspartate potential was visible even when high ejecting currents (up to 2 μA) were used and rapid ($T_{max} \leqslant 100$ ms) glutamate potentials were obtained at the same site. We found no relation between the glutamate T_{max} and the amplitude of the aspartate potential, and no aspartate response could be evoked from sites where glutamate was ineffective.

Interaction experiments

In Figure 6c, aspartate was pulsed at various times after a 'conditioning' glutamate pulse in order to test for mutual interaction. With decreasing pulse interval (550 to 50 ms) the aspartate potential almost doubled in amplitude while its T_{max} and half-decay time ($T_{1/2}$) decreased (amplitudes were measured with respect to the extrapolated time course of the preceding pulse); when pulsed simultaneously, the glutamate potential was enhanced by 70% (cf. Figure 6a). Similarly, when a small dose of glutamate was pulsed at various times during an aspartate potential (Figure 6d) the amplitude of the glutamate potential was also increased (up to 90%) with decreasing pulse interval (the time course of enhancement coinciding roughly with the time course of the aspartate potential) although with little change in T_{max} or $T_{1/2}$; depression of a test pulse following a 'conditioning' pulse of glutamate or aspartate was never observed with the ejecting currents used.

In Figure 6a, the potentiation factor for the coincident glutamate/aspartate potential was 1.9. If the level of aspartate depolarization attained at the time of the peak of the control glutamate potential was expressed in terms of an equivalent glutamate concentration obtained from the glutamate 'dose'-response relation at that site (not illustrated), the predicted amplitude of the coincident potential was close to the observed value. These experiments therefore failed to reveal the glutamate/aspartate interaction seen with bath-appli-

Table 1 Effects of bath-applied aspartate (50 μM; 2 min contact) on amplitude (mV), time to peak (T_{max}; ms) and half-decay time ($T_{1/2}$; ms) of the iontophoretic glutamate potential

	Control glutamate potential			*% change in amptitude and time course of glutamate potential in presence of aspartate*		
	mV	T_{max}	$T_{1/2}$	Amplitude	T_{max}	$T_{1/2}$
1	2.5	67	62	+25	+14	+12
2	2.8	48	62	+31	n.d.	+42
3	1.7	133	150	+17	+8	+27
4	1.9	21	40	+21	n.d.	+15
5	1.3	55	71	−8	n.d.	+11
6	3.1	57	69	−42	n.d.	+20
			Mean	+7.3		+21.2*
			± s.d.	±27.7		±11.8

*The increase in $T_{\frac{1}{2}}$ was significant ($P < 0.01$).
Results are from 6 experiments on different preparations. + = increase; − = decrease; n.d. = no measurable difference.

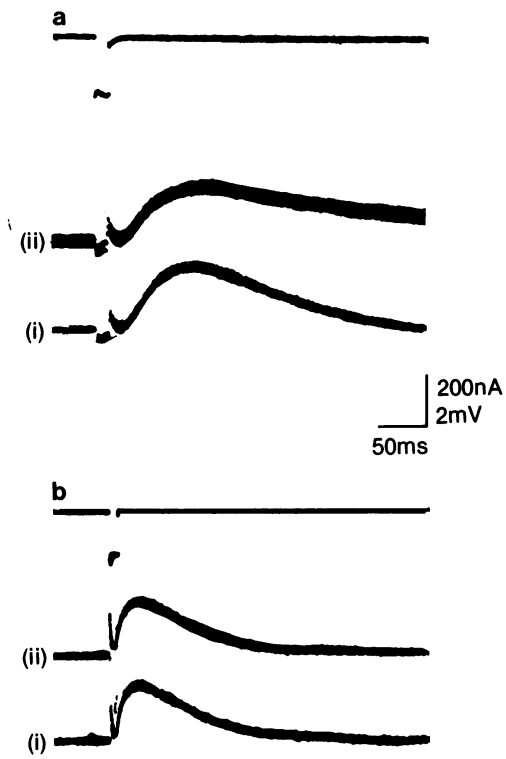

Figure 7 Effect of bath-applied aspartate (0.5 mM) on the glutamate potential; (a) and (b) were recorded at the same sensitive site, but in (b) the potential was better localized. a: (i), control 'diffuse' potential (T_{max} = 102 ms; $T_{\frac{1}{2}}$ = 124 ms); (ii), 30, 60 and 120 s respectively after applying aspartate (3 traces superimposed), note large increase in $T_{\frac{1}{2}}$ (60%). The recovery potential after 8 min wash was exactly superimposed on control b: (i), control 'localized' potential (T_{max} = 30 ms; $T_{\frac{1}{2}}$ = 60 ms); (ii), 30 and 120 s respectively after aspartate (2 traces superimposed), note absence of effect on T_{max} or $T_{\frac{1}{2}}$. In both (a) and (b), aspartate depolarized the membrane by 4 mV.

cations, and any potentiation of iontophoretic aspartate potentials by glutamate (and *vice versa*) could probably be largely explained by a summation of agonist concentrations interacting with a non-linearly responding receptor system (cf. Dudel, 1975).

Effect of bath-applied agonists on glutamate potential

Crawford & McBurney (1977b) observed a potentiation and prolongation of the excitatory junction currents on crab muscle in the presence of bath-applied aspartate (or glutamate) and attributed this to an inhibition of the transmitter (glutamate?) uptake process. However, these workers did not test the effect of other depolarizing agonists under the same conditions. Table 1 summarizes the results from 6 experiments in which we studied the effect of bath-applied aspartate on the amplitude and time course of the iontophoretic glutamate potential. Initially, aspartate (1 mM) was bath-applied repeatedly until responses had stabilized, then the glutamate potential amplitude was chosen to be about half-maximal. Checks for movement artefacts were made by flushing with normal lobster saline solution and only results showing recovery potentials of amplitude greater than 90% of control were accepted.

Aspartate (50 μM) had no detectable effect on membrane potential or resistance, but it reversibly increased the amplitude and prolonged the half-decay time of the glutamate potential, with little effect on T_{max} (compare the effect of iontophoretically-applied aspartate). Lower concentrations of aspartate or subthreshold concentrations of glutamate (< 30 μM) had no effect on the glutamate potential. Table 1 shows no obvious relation between the % increase in amplitude and % increase in $T_{1/2}$ of the glutamate potentials in aspartate-containing solution and the T_{max} of the control potential. However, when the effect of aspartate was tested on a poorly-localized and then a well-localized glutamate potential at the *same* junction, the amplitude and time course of the former was more markedly affected (Figure 7). Similar results were obtained with approximately equieffective doses of glutamate (50 μM), domoate (50 μM) or kainate (0.5 mM) although relative to aspartate, these agonists induced a smaller change in the time course of a diffuse potential; quisqualate (1 μM) suprisingly had no obvious effect on the amplitude or time course.

Discussion

Experiments with bath-applied amino acids

The depolarizing action of bath-applied aspartate on lobster muscle was weaker than that of glutamate on a molar basis. Similar findings have been obtained on crab muscle (Crawford & McBurney, 1977a) but not on the crayfish (Dudel, 1977) where aspartate was apparently ineffective even at a dose of 100 mM. The initial decline in successive aspartate responses and the ability of a 'conditioning' dose of glutamate to enhance subsequent aspartate effects was a novel finding that has not been reported on other crustacean preparations (Crawford & McBurney, 1977a; Dudel, 1977). Clearly, if such an effect is ignored it can lead to an overestimate of aspartate potency relative to glutamate and studies of glutamate/aspartate synergism (Shank & Freeman, 1975) can become complicated. One possible explanation is that aspartate responses were very prone to desensitization and that

glutamate was decreasing this process. However, we observed that successive aspartate responses reached a plateau level rather than declined continuously; we also failed to restore them to the initial amplitude even after a 30 min wash period. No evidence of desensitization to aspartate was seen by applying repeated iontophoretic pulses of this amino acid. It is interesting to note that the 'fading' of bath-applied aspartate responses progressively decreased until restored by a dose of glutamate (Figure 1). If this fading were indeed due to desensitization, then our findings would argue against a role of glutamate in reducing aspartate desensitization.

An alternative hypothesis is that aspartate was exerting a direct agonist effect on the fibre membrane as well as an indirect effect via release of glutamate from intracellular stores. These stores might have been progressively depleted by successive applications of aspartate resulting in a plateau response level, presumably due to residual agonist activity; subsequent applications might then have replenished glutamate stores and thereby enhanced aspartate effects. Glutamate is known to be actively accumulated by nerve/muscle preparations of the lobster (Iversen & Kravitz, 1968) and the competition by aspartate for this uptake could conceivably reduce the recapture of glutamate leaking from nerve and muscle tissues (see Daoud & Miller, 1976; Bowery, Brown, Collins, Galvan, Marsh & Yamini, 1976). It is of interest that only aspartate responses showed this phenomenon since responses to kainic, domoic or quisqualic acid did not progressively decline, nor were they influenced by prior application of glutamate. Little is known about the active transport of these amino acids; however, with kainic acid no interference of glutamate transport by crustacean or mammalian preparations has been found (Shinozaki & Shibuya, 1974b; Roberts & Watkins, 1975).

A third hypothesis is that the reduction in aspartate effect was due to progressive inactivation (neuronal uptake?) of this amino acid and that glutamate was somehow competing with aspartate for this process to produce a temporary increase in the aspartate response (glutamate is a good inhibitor of aspartate uptake, see Balcar, Borg & Mandel, 1977). However, if this were the case, then the aspartate responses after glutamate would be expected to be *slower* in offset as well as larger, due to delayed removal of aspartate from the receptor regions. We found that these enhanced responses became *faster* in onset and offset rather than more prolonged (Figure 1); only direct measurements of amino acid transport will clarify these possibilities.

Iontophoretic experiments

Some interesting points emerged from the study of iontophoretic glutamate and aspartate potentials. Firstly, in contrast with bath-applied aspartate responses, successive aspartate potentials did not progressively decrease in amplitude and were enhanced by a 'conditioning' dose of glutamate only when these two agents were pulsed within about 200 ms of each other. Moreover, when aspartate and glutamate were pulsed simultaneously, the response was not greater than that expected for a glutamate dose (equieffective with aspartate) combined with a test glutamate dose. A similar explanation could also account for the potentiation of glutamate potentials by iontophoretic aspartate as seen by Shank *et al.* (1977). It is also worth noting that Crawford & McBurney (1977a) applied glutamate and aspartate to crab muscle by focal superfusion, which might explain why a 'conditioning' glutamate dose did not apparently influence aspartate responses on this preparation.

Secondly, large currents were required to evoke an aspartate potential which, whenever present, had a slower time course than the glutamate potential, despite the similar transport number of these amino acids (Gent, Morgan & Wolstencroft, 1974). If aspartate were a weak glutamate receptor agonist, then a large aspartate ejection would be required to evoke a detectable response, the time course of which may be expected to be slow due to contributions from the wide area affected by the released aspartate. Alternatively, if distinct aspartate-sensitive receptors exist, their sparse location at the glutamate-sensitive areas might account for the present results.

The enhancement and prolongation of the glutamate potential by bath-applied aspartate accords with the findings of Crawford & McBurney (1977b); however, we feel that inhibition of glutamate uptake by aspartate cannot solely explain this result since a similar effect was seen with kainic acid, which does not affect glutamate uptake (Shinozaki & Shibuya, 1974b; see also Takeuchi & Onodera, 1975); therefore, in aspartate (or kainate)-containing solutions iontophoretically-applied glutamate activates a wider region of the receptive area. It has been suggested that kainate acts only on extrajunctional glutamate receptors (Shinozaki & Shibuya, 1974b; Takeuchi & Onodera, 1975). By analogy, it is possible that bath-applied aspartate also acted extrajunctionally; summation of aspartate and glutamate at these sites could therefore influence the shape of the junctional glutamate potential. This is supported by two findings: (i) in aspartate solution the more diffuse glutamate potential was markedly prolonged and (ii) the influence of aspartate on the shape of the glutamate potential was larger when aspartate was bath-applied rather than iontophoretically ejected onto glutamate-sensitive sites. Since bath-application of glutamate, domoate and kainate (but not quisqualate) also affected the time course of the diffuse glutamate

potential, then it would appear that extrajunctional sites are sensitive to a number of agonists, aspartate being the more potent among them. Our results would also explain the absence of effect of bath-applied aspartate on the amplitude of the excitatory junctional potentials of lobster muscle (Shank *et al.*, 1975) because of the very localized nature of these potentials.

In conclusion, we propose that aspartate, when bath-applied to lobster muscle, may produce variable responses depending mainly on the amount of glutamate released from the tissue. From iontophoretic experiments we found that aspartate action is not influenced by a previous application of glutamate and we suggest that the maximum sensitivity of lobster fibres to aspartate might be at extrajunctional membrane regions.

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