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The amnesic shellfish poison domoic acid enhances neurotoxicity by excitatory amino acids in cultured neurons

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Summary. A recent episode of human intoxication by cultured mussels containing a rare excitatory amino acid named domoic acid, received particular attention for its neurological implications. The intoxication produced neurological problems, such as headache, confusion, and loss of memory, particularly severe at times. Neuronal damage was found in the hippocampus and amygdala of four patients. We now report that in neuronal cultures the neurotoxicity of a domoic acid-containing mussel extract is the result of domoic acid potentiation of the excitotoxic effect of glutamic acid and aspartic acid present in high amounts in mussel tissue. Moreover, we show that subtoxic concentrations of domoic acid are sufficient to potentiate glutamic acid and aspartic acid neurotoxicity. We present evidence suggesting that the neurotoxic synergism may be due to a reduction of Mg^{++} block at the NMDA receptor-associated channel, following activation of NON-NMDA receptors by domoic acid.

Keywords: Amino acids – Domoic acid – Toxic mussels – Toxic synergism – Excitatory amino acids – Biotoxins – Environmental neurotoxins

Introduction

Several kinds of food contain high amounts of common excitatory amino acids (EAAs), such as aspartic acid and glutamic acid. Occasionally, rare EAAs may be present, as it occurred with domoic acid (DA) in Canadian cultured mussels. DA is a tricarboxylic amino acid possibly produced by the unicellular alga *Nitzschia pungens*, and accumulated by the cultured mussels, Wright (1990). The presence of DA in the food had never been demonstrated before the Canadian episode of intoxication by mussels which occurred during the period

November–December 1987. 107 Canadians suffered for gastrointestinal problems after eating cultured mussels and were hospitalized, Perl (1990). In several cases neurological problems followed the ingestion of the mussels. Neurological symptoms included headache, confusion, disorientation and loss of memory. The syndrome was named *amnesic shellfish poisoning*. Several people suffered long-lasting memory loss and five elderly people who suffered the most serious neurological problems, died at different times after the acute episode of intoxication. When autopsic analysis of the brain was performed, it revealed neuronal damage in the amygdala and hippocampus, Teitelbaum (1990). DA has been classified as an excitatory amino acid (EAA) based on earlier studies, Biscoe (1975). Since then, a few studies in mammals, Wood (1982), Wright (1990), Zaczek (1982) have confirmed that DA action occurs through the NON-NMDA subtype, Stevens (1986) of EAA receptors. Cultured mussels are known to contain relevant amounts of the dicarboxylic aminoacids aspartic acid and glutamic acid. These two excitatory amino acids may also be responsible for neurotoxicity, Choi (1988), Collingridge (1989). We have investigated the biochemical and toxicological effects of a DA-containing toxic-mussel extract, using primary cultures of cerebellar neurons. This experimental system has already been useful for the investigation of the biochemical effects of EAAs, McCaslin (1987), Nicoletti (1986), Novelli (1987 a), Novelli (1987 b), Schousboe (1985) and the conditions controlling EAAs neurotoxicity, Favaron (1988), McCaslin (1988), Novelli (1988).

Materials and methods

Cell culture

Primary culture of rat cerebellar neurons were prepared as described previously, Novelli (1988). Briefly, cerebella from 8-day-old pups were dissected, cells were dissociated and suspended in basal Eagle's medium with 25 mM KCl, 2mM glutamine, 100 μg/ml gentamycin and 10% fetal calf serum. Cells were seeded in poly-L-lysine coated (5 μg/ml) 35 mm dishes at 2.5×10^5 cells/cm^2 and incubated at 37°C in a 5% CO_2, 95% humidity, atmosphere. Cytosine arabinoside (10 μM) was added after 20–24 h of culture to inhibit the replication of non-neuronal cells. After 8 days in vitro, morphologically identifiable granule cells accounted for more than 95% of the neuronal population, the remaining 5% being essentially GABAergic neurons as already shown, Nicoletti (1986). Astrocytes did not exceed 3% of the overall number of cells in culture as previously reported, Nicoletti (1986). Cerebellar neurons were kept alive for more than 40 days in culture (DIC), by replenishing the growth medium with glucose every 4 days and compensating for lost amounts of water, due to evaporation, Novelli (1990).

Neurotoxicology

Primary cultures of cerebellar granule cells were used for neurotoxicological studies between 10–30 days in culture. Drugs were added in the growth medium for 24 hours. After 24 hours of exposure to the drugs, the growth medium was removed and cultures were incubated for 5 min. with 1 ml incubation buffer containing (in mM): 154 NaCl; 5.6 KCl; 5.6 glucose; 8.6 Hepes; 1 $MgCl_2$; 2.3 $CaCl_2$; pH 7.4, to which the vital stain fluorescein diacetate (5 μg/ml) was added. The staining mixture was then aspirated and replaced with incubation buffer. The neuronal culture were then examined for neurotoxicity. Under phase contrast microscopy, live neurons were light refractive and possessed long neurites, dead neurons were

reduced in size to the nucleus and neurites were absent. Under fluorescence, live neurons in culture showed a bright green color in the cell body and the neurites, while dead neurons did not retain any fluorescein diacetate and the nucleus could be stained in red by short exposure to ethidium bromide, Novelli (1988), Novelli (1990). Photographs of randomly selected culture fields were taken and live neurons were counted independently by two of the authors and the results were averaged. Results were then related to total culture population.

Biochemistry

Intracellular cGMP concentration was determined as previously reported, Novelli (1987 a). Briefly, cultures were washed twice with 1 ml prewarmed (37°C) incubation buffer containing (in mM): 154 NaCl; 5.6 KCl; 5.6 glucose; 8.6 Hepes; 1 $MgCl_2$; 2.3 $CaCl_2$; pH 7.4. ($MgCl_2$ was omitted when indicated). Dishes were incubated at 37°C for 10 min with 1 ml fresh incubation buffer and for an additional 20 min with a second 1 ml fresh incubation buffer. Drugs were added at the end of the 20 min incubation period for 1 min. Antagonists were added 1 min. before the agonists. Incubation was stopped by aspiration of the solution and addition of 1 ml $HClO_4$ (0.4 N). After neutralizing the perchlorate extract, cGMP content was determined by radioimmunoassay. Protein content was determined on the membrane pellet from the same sample.

Data presentation and analysis

Results are presented as indicated in each figure. Statistical analysis has been performed by Student *t*-Test.

Materials

DA was purchased from Diagnostic Chemicals Ltd, West Royalty Industrial Park, Charlottetown, PEI, C1E1B0, Canada; (+)-10,11-dihydro-5-methyl-5H-dibenzo-[*a*, *d*]-cyclohepten-5,10-imine hydrogen maleate (MK-801) was a gift from Merck Sharp and Dohme, D(−)-2-Amino-5-phosphonovaleric acid (APV) and quisqualic acid were purchased from Cambridge Research Biochemicals, CNQX was purchased from Tocris Neuramin. All other drugs were from Sigma.

Mussels extract

Mussels extracts have been prepared from cultured mussels collected at the time of the Canadian episode of intoxication. Toxic and non-toxic cultured mussels, were collected. Non-toxic mussels were collected from an area not infested by DA-producing algae, geographically different from the one where toxic mussels were harvested. After the harvest, mussels were shucked, homogenized in a Sorvall Omnimixer at full speed setting, and the homogenate was lyophilized. For the determination of DA, lyophilized mussels (245 mg) were suspended in distilled water (30 ml) and kept at 70°C for 10 min with stirring. After cooling at room temperature, the mixture was poured into absolute ethyl alcohol (60 ml) and then centrifuged in a bench top centrifuge. The clear supernatant was evaporated to about 15 ml in a rotatory evaporator at room temperature and the residue was adjusted to 25 ml in a volumetric flask. An aliquot of this solution was diluted 1:5 and used for the determination of DA by HPLC, as previously described by Quilliam, M. A., Sim, P. G., McCulloch, A. W. and McInnes, A. G. "Determination of domoic acid in shellfish tissue by high-performance liquid chromatography". National Research Council of Canada, Atlantic Research Laboratory Technical Report 55, (1988), NRCC 29015. In brief, a Whatman Partisil ODS II, 10 μm column (25 cm × 4.6 mm) was eluted (1 ml/min) by aqueous 12.5% acetonitrile, adjusted to pH 2.5 with phosphoric acid. The detectors were a Schoefel variable wavelength detector, set at 242 nm, and an HP 8452A diode array spectrophotometer, scanning from 200 to 300 nm every 5 sec. Sensitivity of the method in mussel tissue was

approx. 13 nmol of DA/g wet weight. Free amino acids were extracted by 0.1 N HCl, derivatized by OPA and measured by HPLC by A. Stehouwer, Research and Productivity Council, Fredericton, N. B., Canada, according to the method of Hill (1979). All the determinations were reported to dry weight (g) and a ratio wet/dry weight = 10 has been assumed, based on a fresh mussel tissue density of 1.03 g/ml.. For tissue culture experiments, 500 mg of lyophilized mussels were resuspended in 50 ml distilled water, heated at 70°C for 10 min, then centrifuged at 30,000 × g for 1 hour at 4°C. Supernatant was lyophilized and the sample was then reconstituted with distilled water (6.4 ml). The pH of the solution was adjusted to 7.4.

Results

EAA content in the extract from the Canadian toxic mussels that produced the human episode of intoxication in 1987 (TE) was determined and results are reported Fig. 1. Aside from the presence of DA in TE, total EAA content in TE was similar to the values reported in the literature (Fig. 1). We determined the concentration-dependent neurotoxicity curves produced by purified DA, glutamic acid (GA) and aspartic acid (AA) (Fig. 2). We included D-aspartic acid (D-AA), that has been reported to represent approximately 40% of total AA in mussel tissue, Felbeck (1987). D-AA and L-AA produced a very similar concentration-dependent neurotoxicity curve, where 50% neurotoxicity was produced

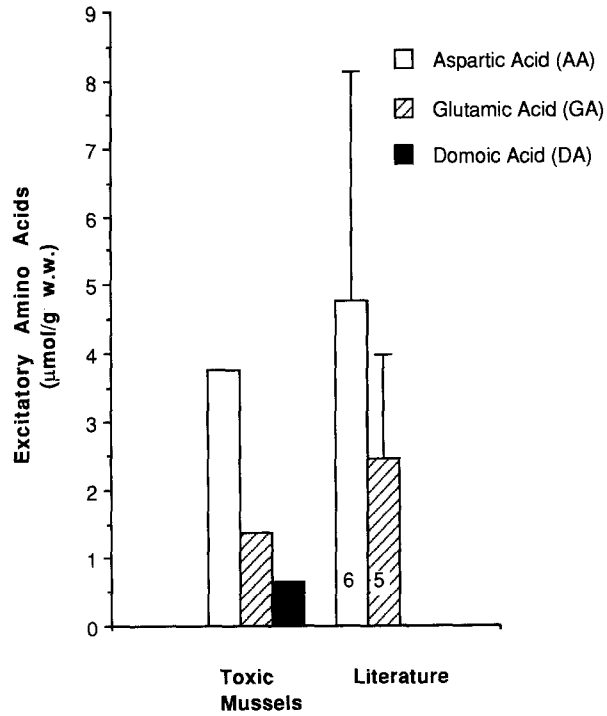

Fig. 1. Excitatory amino acid content in cultured mussels. Detection limit for DA in mussel tissue was ~13 nmol/g wet weight (see methods). Excitatory amino acid content in mussels, as calculated from literature values [7, 11, 13, 28], is expressed as average ± SD. The number of data included in the calculation is reported in the corresponding column. For determinations related to dry weight, a ratio wet weight/dry weight = 10 has been assumed from a fresh mussel tissue density of 1.03 g/ml

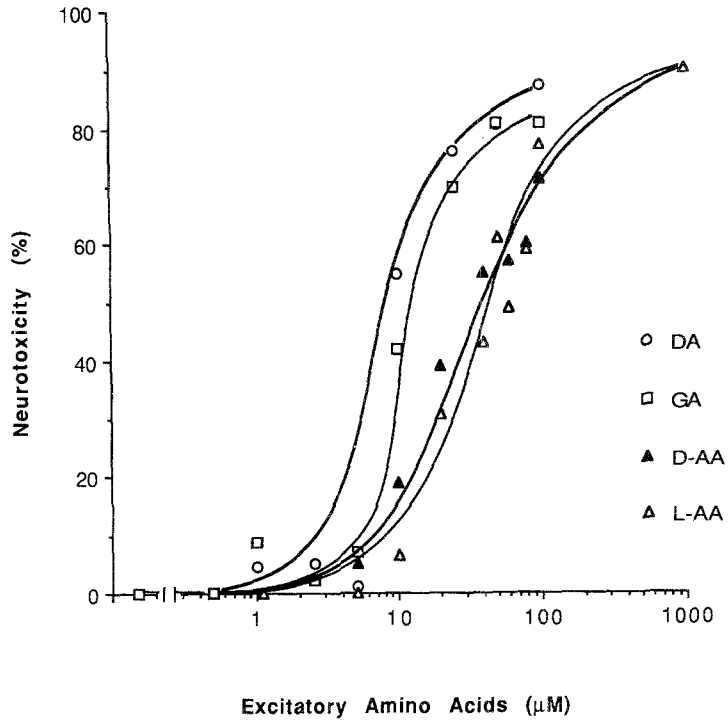

Fig. 2. Concentration-dependent neurotoxic effect of domoic acid (*DA*), glutamic acid (*GA*), D-aspartic acid (*D-AA*) and L-aspartic acid (*L-AA*) in cerebellar granule cells in primary culture. The number of live neurons after 24 hours exposure to the drugs was calculated as described in the methods and then divided by the number of live neurons in control cultures that were not treated with any drug. % Neurotoxicity was calculated as 100-% live neurons. Data reported are from two to three experiments. Each point is the average of four to six determinations. SD was ~10–20% of the mean

by ~35 μM of either D-AA or L-AA (Fig. 2). DA and GA produced 50% neurotoxicity at ~8 μM and ~13 μM respectively (Fig. 2).

In order to characterize TE neurotoxicity, we first characterized pharmacologically the neurotoxicity of each EAA in the extract (table I). DA (20 μM) neurotoxicity was fully antagonized by quisqualic acid (QA, 25–100 μM), an EAA agonist at the NON-NMDA receptor, Stevens (1986), Collingridge (1989) capable of antagonizing the effects of kainic acid, Gallo (1989), McCaslin (1988) (an other EAA structurally similar to DA, Biscoe (1975)). DA neurotoxicity was fully antagonized also by the quinoxalinedione CNQX (10–50 μM), a NON-NMDA receptor antagonist, Collingridge (1989) (data not shown), while it was not prevented by competitive and non-competitive antagonists of EAA at the NMDA-receptor such as APV (1 mM) and MK-801 (1 μM) respectively, Collingridge (1989) (Table 1). Neurotoxicity by GA (40 μM) and AA (200 μM) was fully antagonized by APV(1 mM), MK-801 (1 μM), and was unaffected by QA (Table 1). CNQX (50 μM) reduced neurotoxicity by AA and GA, possibly by antagonizing the action of glycine, Collingridge (1989). Neurotoxicity by TE was only reduced by either QA, or APV or MK-801 when used alone (Table 1), although a combination of QA plus APV or QA plus MK-801 could fully

Table 1. Neurotoxicity by EAAs and mussel extracts in cultured cerebellar neurons

Agonist	Antagonist					
	none	APV	MK-801	QA	APV + QA	MK-801 + QA
	(number of live neurons/1000)					
none	966 ± 34	981 ± 13	991 ± 4	925 ± 42	979 ± 12	954 ± 40
DA	88 ± 7	378 ± 38	189 ± 16	943 ± 36	nd	nd
AA	150 ± 35	950 ± 30	897 ± 75	200 ± 70	nd	nd
GA	102 ± 20	975 ± 27	985 ± 22	160 ± 40	nd	nd
TE	94 ± 24	500 ± 100	460 ± 10	304 ± 14	1040 ± 153*	870 ± 47*
CE	415 ± 93	1017 ± 22**	875 ± 108**	422 ± 113	nd	nd

Toxic mussel extract (*TE*) was diluted to a final 1.3% in the growth medium (~58 μM total EAAs). An extract from DA-free mussels (*CE*) of known EAA content, was similarly diluted to a final 1.3% in the growth medium (~52 μM total EAAs). Glutamic acid (*GA*) was used at 40 μM. Aspartic acid (*AA*) was used at 200 μM. Domoic acid (*DA*) was used at 20 μM, APV at 1 mM, MK-801 at 1 μM, quisqualic acid (*QA*) at 25–100 μM. APV, MK-801, QA were added 1 min. before agonists. The number of live neurons after 24 hours exposure to the drugs, was calculated as described in the methods. Data are expressed as the mean ± SD from 3 independent experiments performed in duplicate or triplicate. * = $P < 0.01$ vs TE-none. ** = $P < 0.01$ vs CE-none. Statistical significance has been tested by Student's *t*-test. *nd* not done

antagonize TE neurotoxicity (Table 1). When CNQX was substituted for QA, similar results were obtained (data not shown). These results indicate that TE neurotoxicity for cerebellar neurons in culture is due exclusively to the presence of both NON-NMDA and NMDA receptor agonists in TE. As compared with TE, a mussel extract from DA-free mussels (CE) was less neurotoxic. Moreover, CE neurotoxicity was fully antagonized by APV and MK-801, while unaffected by QA (Table 1), indicating the exclusive presence of NMDA receptor agonists in CE.

TE produced 50% neurotoxicity at ~9 μM EAA (Fig. 3). At this concentration of EAA in CE, no neurotoxicity was observed (Fig. 3). Moreover, when comparing TE and DA neurotoxicity, 50% neurotoxicity was achieved at ~1 μM DA concentration within TE (Fig. 3), while a similar neurotoxic effect required ~8 μM for purified DA (Fig. 2), suggesting a synergistic interaction between DA, AA and GA. In order to verify that DA was the only substance responsible for the increased neurotoxicity of TE as compared to CE, we added

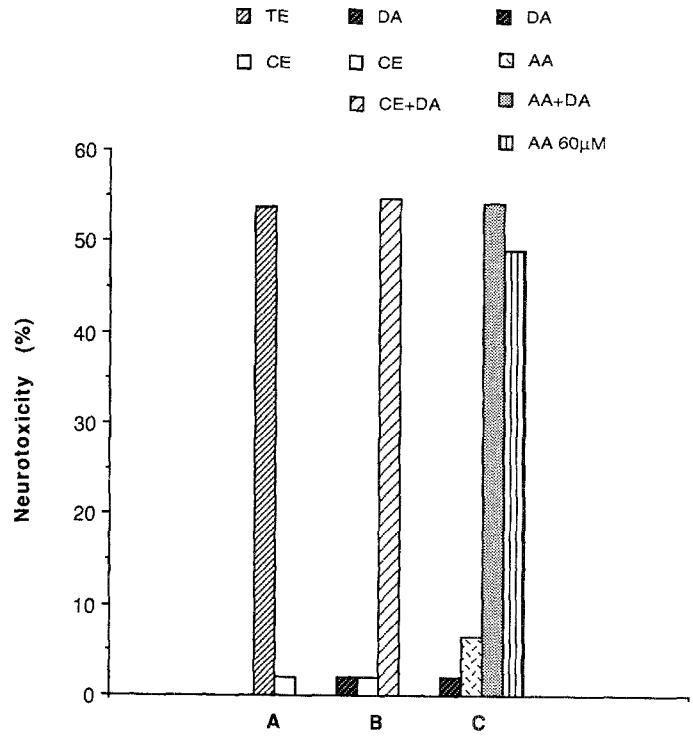

Fig. 3. Subtoxic concentrations of domoic acid potentiate NMDA receptor-mediated neurotoxicity. **A** Toxic mussel extract (*TE*) was diluted to a final concentration of 9 μM EAAs in the growth medium For comparison, a non-toxic mussel extract (*CE*) was diluted to a similar final concentration of EAAs. **B** A subtoxic concentration of purified DA (2–5 μM) was added to a dilution of CE in neuronal growth medium providing a subtoxic final concentration of EAA equal to 4–7 μM. **C** DA at a subtoxic concentration of 2 μM, was added to a subtoxic concentration of AA (10 μM). For comparison, neurotoxicity by AA at 60 μM, is reported. Results from at least three experiments performed in duplicate, are reported. SEM was ~10% of the mean. CE contained (in μmol/g wet weight): aspartic acid = 2.05; glutamic acid = 3.15; domoic acid = not detectable

non-toxic concentrations of DA (2–5 μM) to a non toxic dilution of CE, corresponding to ~4–7 μM EAA, giving a final concentration of 9 μM total EAA. Such addition of DA to CE produced a neurotoxic effect of magnitude comparable to the one produced by TE (Fig. 3). In order to prove that the potentiation of the action of NMDA receptor agonists by DA was not due to any unidentified component of the mussel extract, we repeated the experiment using purified EAAs (Fig. 3). We chose AA because it is the most abundant EAA in mussel tissue and possess the weakest neurotoxicity as shown in Fig. 2. The addition of a subtoxic concentrations of DA (2 μM) to a subtoxic concentration of AA (10 μM), produced a neurotoxic effect of magnitude comparable to the one produced by 60 μM AA (Fig. 3).

We then attempted to clarify the mechanism leading to a potentiation of CE neurotoxicity when DA is present. The activation of EAA receptors was investigated by determining intracellular cGMP formation, a reliable index of EAA receptor activation, McCaslin (1987), Novelli (1987 b), Novelli (1988). We found that TE was a potent stimulator of cGMP formation (Fig. 4), although a concentration of purified DA (2 μM), comparable to the concentration of DA within TE, produced only a small increase in cGMP formation (Fig. 4) CE (up to 80 μM EAAs) was almost ineffective in elevating cGMP levels in cerebellar granule cells in primary culture when Mg^{++} (1mM) was present in the incubation buffer (Fig. 4). However, CE at concentrations of EAA as low as 8 μM, promoted a large increase in cGMP intracellular levels when Mg^{++} was removed (Fig. 4), as expected for the NMDA receptor agonists present in CE, Novelli (1987 b). Thus, low, subtoxic, concentrations of DA (2–2.5 μM) producing a small (~20% of maximal) but significant stimulation of cGMP formation in the presence of Mg^{++} (Fig. 4), when added to CE in the presence of Mg^{++}, induced cGMP formation to the same extent as the removal of Mg^{++} (Fig. 4). Similar results were obtained by using purified EAAs (data not shown). DA-mediated cGMP synthesis was independent of the presence of 1 mM extracellular Mg^{++} in the incubation buffer and was selectively antagonized by either QA (25 μM) or CNQX (10 μM) (data not shown). QA did not affect GA stimulation of cGMP synthesis, obtained in the absence of extracellular Mg^{++} (data not shown).

Discussion

Our results show that excitatory amino acids of potential neurotoxicity can be present in some kinds of human food. The presence of EAAs in mussel tissue has been already reported, Felbeck (1987), Hoyaux (1976), Livingstone (1979), Shumway (1977). However, DA, a tricarboxylic amino acid, well known in the Japanese pharmacopeia for its antihelmintic properties, Daigo (1959), and used as a pesticide, Maeda (1984), is not usually present in mussels and appeared suddenly in the mussels cultured in Canada at the time of the episode of intoxication. Besides the presence of DA, and to a minor extent of some weaker DA isomers, amino acid analysis of TE revealed that AA and GA were present in concentrations comparable to those reported in the literature for cultured mussels. All the other amino acids with potentially toxic action such as cysteine,

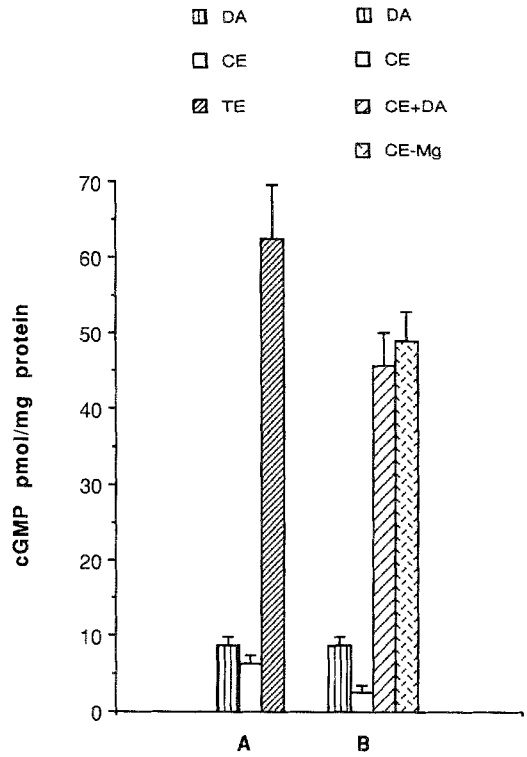

Fig. 4. Domoic acid relieves the Mg^{++} block at the NMDA receptor-associated channel Intracellular concentration of cGMP was measured following 1 min. stimulation of neuronal cultures with a toxic extract (*TE*), a control extract (*CE*), and purified domoic acid (*DA*). **A** TE and CE were diluted to produce a final concentration of 20 μM EAAs in the incubation buffer. DA was used at 2.5 μM, a concentration similar to the one present in TE. cGMP levels in unstimulated cultures was equal to 0.7 $\pm$ 0.06 pmol/mg protein, in the presence of Mg^{++} (1 mM), and was subtracted. **B** CE (8 μM) was tested for stimulation of cGMP formation both in the presence (*CE*) and in the absence of extracellular Mg^{++} (1 mM) (*CE-Mg*). cGMP levels in unstimulated cultures in the absence of Mg^{++} was equal to 1.6 $\pm$ 0.6 pmol/mg protein, and was subtracted. Purified DA (2.5 μM) was tested alone and in association to CE, in the presence of extracellular Mg^{++}. Maximal stimulation of cGMP formation by DA was obtained at 7.5– 10 μM and was equal to 77 $\pm$ 4.6 pmol/mg protein. Data are reported as the mean $\pm$ SD of at least three experiments

Olney (1990) were present in CE and TE to a similar concentration (data not shown). No differences between CE and TE were found in respect to their ionic content of zinc, magnesium, cadmium, cobalt, mercury, vanadium, ... etc (data not shown). The potentiation of CE neurotoxicity we obtained by adding a subtoxic concentration of purified DA to CE, suggests that DA may be the only compound responsible for the increased neurotoxicity of TE compared with CE. Our results show that DA, acting at the NON-NMDA-type of EAA receptor, can be a potent neurotoxin for cerebellar granule cells in primary culture. Indeed, our data indicate that subtoxic concentrations of DA may still produce dramatic neuronal damage when in association with subtoxic concentrations of EAAs acting at the NMDA receptor. In the same experimental system, it has been shown that neurotoxicity by GA and NMDA receptor agonists is depen-

Table 2. Calculation of a synergism factor (*SF*) for the potentiation of NMDA receptor-mediated neurotoxicity by domoic acid

Neurotoxin	50% neurotoxicity (μM)	SF
AA	40.4	—
AA + DA	11.6	3.5
CE	30.6	—
CE + DA	9.4	3.3

Values are calculated from best fitting curves of 1) AA, 2) AA + DA, 3) CE and 4) CE + DA neurotoxicity data (Fig. 2, and data not shown). For AA + DA neurotoxicity curve, DA was used at subtoxic concentrations of 1 and 2 μM in combination with variable subtoxic concentrations of AA from 2 to 10 μM. For CE + DA neurotoxicity curve, DA was used at 2–5 μM in combination with increasing amounts of CE. Best fitting curves of neurotoxicity data (NT) were: 1) $\text{Log[AA]} = 0.8849878 + 0.014437\ \text{NT}_{\text{AA}}$; 2) $\text{Log[AA + DA]} = 0.7585031 + 0.0060793\ \text{NT}_{\text{AA+DA}}$; 3) $\text{Log[CE]} = 0.9176158 + 0.011355\ \text{NT}_{\text{CE}}$; 4) $\text{Log[CE + DA]} = 0.6898769 + 0.0056162\ \text{NT}_{\text{CE+DA}}$.

dent upon the reduction of the Mg^{++} block at the NMDA receptor, Novelli (1988). Thus, our biochemical data on cGMP stimulation suggest that subtoxic concentrations of DA acting at the NON-NMDA receptor may enhance NMDA agonist-mediated neurotoxicity by reducing the Mg^{++} block at the NMDA-receptor-associated channel.

The synergistic interaction between DA and NMDA receptor agonists may be quantified considering the concentration of AA and AA + DA producing 50% neurotoxicity. The ratio [AA]/[AA + DA] may represent a synergism factor (SF), which mimimum value tend to one as the effect of DA tend to zero. Thus, our data in Table 2 show that SF is ~ 3. Similarly, following exposure of neuronal cultures to a control mussel extract, either in the absence or in the presence of a subtoxic concentration of DA, the concentration of CE and CE + DA producing 50% neurotoxicity, may be considered. Our results, reported in Table 2, indicate a significant SF of ~ 3 when DA is present in CE.

In mammalian central nervous system, GA is now considered a potent endogenous excitotoxin, Choi (1988), Collingridge (1989). The neurotoxic effects of systemically administered GA, an excitatory amino acid often present in large amounts in human diet, Skurray (1988) have been shown since 1969, Olney (1969). Cases of intoxication by chinese food have been attributed to the high content in GA, Allen (1981), Schaumburg (1969), which has been calculated by Allen and Baker at 1.7 mmol/100 g, Allen (1981). In mussels, the combined concentration of GA and AA reported in the literature, Felbeck (1987), Hoyaux (1976), Livingstone (1979), Shumway (1977), averages at 0.74 mmol/100 g fresh weight, although it may vary from 0.3 to 1.62 mmol/100 g fresh weight. It must also be noted that D-AA may represent $\sim 40\%$ of total AA in mussel tissue, Felbeck (1987), and favored by its low cellular reuptake in human body, may

possibly reach the blood brain barrier in higher amounts than the L-isomer. Nevertheless, human cases of intoxication atribuible to mussel's high EAA content have never been reported. These considerations reinforce the idea that DA may have been necessary for the occurrence of neurological problems. Moreover, our data suggest that concentrations of DA that per se would have not been sufficient to cause neurotoxicity, could have induced neurotoxicity in the presence of adequate concentrations of NMDA receptor agonists. The relevance of our results in the explanation of the neurological problems reported during the Canadian epsode of intoxication will need further investigation using different experimental systems. Recent data from behavioral studies in rodents did show that the effects of DA-contaminated mussels were involving only EAA receptors, Glavin (1989), and that low doses of DA in extracts of contaminated mussels were significantly more potent than the same doses of purified DA, Tasker (1991). Thus, this observation may be explained by our finding of a synergistic interaction between EAAs, assuming a significative passage of alimentary-related EAAs through the blood brain barrier.

In conclusion, to the best of our knowledge, this is the first study indicating a role for DA in potentiating the activity of NMDA agonists, and it underlines the potential risk to human health represented by excitatory amino acids as environmental biotoxins.

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