Journal of Neurochemistry Vol. 33, pp. 1309 to 1315
Pergamon Press Ltd 1979. Printed in Great Britain
International Society for Neurochemistry Ltd

EFFECTS OF EXCITATORY NEUROTRANSMITTER AMINO ACIDS ON SWELLING OF RAT BRAIN CORTICAL SLICES[1]

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Abstract—With the single rat brain cortical slice serving as an *in vitro* bio-assay system, the effects of neurotransmitter amino acids (1 mM) on brain swelling, water, sodium and potassium content, inulin space, and lactate production were studied. The putative dicarboxylic amino acid neurotransmitters, L-glutamic acid and L-aspartic acids, greatly increased intracellular brain swelling with increased intracellular Na^+, water content and lactate production, and decreased inulin space and intracellular K^+. Equimolar GABA, taurine, glycine, the putative inhibitory neurotransmitter amino acids, and equimolar α-amino-isobutyric acid had no effect. Brain swelling and intracellular Na^+/K^+ ratios were greatly increased by L-glutamate and L-aspartate at a concentration of 10 mM. However, L-aspartate at these concentrations greatly depleted the K^+ content and lactate production as compared to L-glutamate.

Further studies indicated that only the structural analogs and isomers of the dicarboxylic amino acids possessing two acidic groups and an α-amino group had a similar effect on the induction of brain swelling. Among the analogs of glutamic acid, DL-homocysteic acid and kainic acid had a greater effect on brain swelling, as observed from the total adenosine 5'-triphosphate (ATP) levels and the time-course and dose-response. A biphasic response in lactate production was induced by DL-homocysteic acid and kainic acid, suggesting that these analogs had a neurotoxic effect on cellular metabolism at higher concentrations.

IT IS well known that central neurotransmitters or neuroregulators are released from various nerve tissues upon electrical stimulation, elevated K^+ or hyperosmotic manipulation (CHAN & FISHMAN, 1977; CHAN *et al.*, 1978). The released neurotransmitters are then involved in the mechanism of depolarization by binding to receptor sites on the postsynaptic membrane. It has been shown that glutamate and aspartate increased cellular swelling and decreased the extracellular inulin space in brain slices; lysine, glycine, histidine and valine did not have an effect (BANAY-SCHWARTZ *et al.*, 1974). Furthermore, various excitatory neurotransmitter amino acids cause membrane depolarization and increase permeability of cellular membranes to sodium ions (BRADFORD & MCILWAIN, 1966; CURTIS & WATKINS, 1965; OKAMOTO & QUASTEL, 1970). Perfusion of 10 mM-glutamate, an excitatory neurotransmitter amino acid, into cat brain caused development of edema measured 24 h later (OETTINGER *et al.*, 1976). This intracellular swelling was confirmed morphologically; it was found to involve both neurons and glial structures (MOLLER *et al.*, 1974). These data suggest that excitatory neurotransmitters may be involved in brain edema formation by affecting the permeability of brain membranes *in vivo*. Apart from this possibility, excitatory neurotransmitters also participate in the regulation of cellular metabolism. It has been shown that excitatory amino acids cause the degeneration of neurons in the retina and hypothalamus in infant mice, whereas equimolar glutarate and basic and neutral amino acids had no effect (OLNEY *et al.*, 1971, 1974). Furthermore, L-glutamate and its structural analogs increased oxygen uptake, lactate production, and NAD-levels in rat brain (COX *et al.*, 1977). Kainic acid and homocysteic acid were the most effective analogs affecting both membrane depolarization and cellular metabolism. However, the relationship between the excitatory effects of neurotransmitter amino acids and the brain swelling associated with changes in cellular metabolism requires further investigation. Using single first, rat brain cortical slices as an *in vitro* bioassay system, we have demonstrated previously that cellular (cytotoxic) brain edema induced by white blood cell membrane fractions and polyunsaturated fatty acids is characterized by increases in water content, lactate production, Na^+ content, and by decreases in K^+ content, ATP and energy charge, and extracellular inulin space (CHAN & FISHMAN, 1978; FISHMAN *et al.*, 1977*a*). The present studies report the effects of the excitatory neurotransmitter amino acids L-glutamate, L-aspartate and several structural analogs of these dicarboxylic amino acids on cellular swelling, lactate production, intracellular Na^+ and K^+ content.

[1] This work was supported by N.I.H. Grant NS 14543.

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Abbreviations used: HEPES, N-2-hydroxyethylpiperazine-N-2-ethanesulfonic acid; GABA, γ-aminobutyric acid.

MATERIALS AND METHODS

Sprague–Dawley rats (purchased from Simonsen, Gilroy, CA) weighing 100–200 g were decapitated, with the brain rapidly removed and placed on ice. Single first cortical slices from each hemisphere 40–50 mg in weight and 0.35 mm thick were cut with the pia intact, according to a previously described method (CHAN *et al.*, 1978; FISHMAN *et al.*, 1977*b*). The initial wet weight of each slice was quickly measured with Precision Balance (Federal Pacific Electric Co., Northboro, Mass.), and was then incubated in either 5 ml Krebs–Ringer buffer as control, or in 5 ml Krebs–Ringer containing the experimental compounds at 37°C in aquatherm water shaker (New Brunswick Scientific, New Brunswick, NJ). The incubation time was 90 min in most studies except for a 60 min incubation used in the dose–response studies. Krebs–Ringer media had the following composition (in mM): NaCl, 140; KCl, 3.6; $CaCl_2$, 1.5; KH_2PO_4, 1.4; $MgSO_4$, 0.7; glucose, 10; *N*-2-Hydroxyethylpiperazine-*N*-2-ethane-sulfonic acid (HEPES), 15 at pH 7.4; and the osmolality was 305 mosmol/l. L-glutamic acid, L-aspartic acid, D-glutamic acid, D-aspartic acid, GABA, L-taurine, α-amino-isobutyric acid, L-glycine, L-cysteine, L-cystine, L-cysteic acid, kainic acid, L-pyroglutamic acid, α-methyl-DL-aspartic acid, *N*-methyl-DL-glutamic acid, and HEPES were obtained from Sigma Company, St. Louis, MO. DL-homocysteic acid, *N*-methyl-DL-aspartic acid were purchased from ICN Pharmaceutical, Inc., Plainview, NY. [^{3}H(G)]-inulin, Mw 5,000, 100–500 mCi/g was purchased from New England Nuclear, Boston, MA, and repurified by Sephadex G-25 column chromatography to eliminate fructose fragments prior to use. 1 μCi [^{3}H]-inulin was routinely added to the incubation media for the determination of extracellular inulin space.

After incubation, slices were slightly blotted on an acid-washed glass Petri dish quickly to remove the excess surface-bound water, and the final wet weight was measured. Increased wet weight (%) was calculated as:

$$\frac{\text{final wet weight} - \text{initial wet weight}}{\text{initial wet weight}} \times 100.$$

Cortical slices were then dried at 105°C for 16 h to obtain constant dry weight and the dry weight %, tissue water content and the tissue swelling (%) were measured.

$$\text{Initial or final water content (\%)} = \frac{\text{initial or final wet weight-dry weight}}{\text{initial or final wet weight}} \times 100.$$

$$\text{Tissue swelling (\%)} = \frac{\text{water content (\%) (final-initial)}}{\text{initial water content (\%)}} \times 100.$$

$$\text{Dry weight \%} = \frac{\text{dry weight}}{\text{initial wet weight}} \times 100.$$

Dried slices were extracted with 2N-nitric acid for 16 h, and aliquots were used to determine inulin space (%), intracellular Na^+ and K^+ contents.

$$\text{Inulin space (\%)} = \frac{\text{d.p.m./g brain final wet weight}}{\text{d.p.m./ml medium}} \times 100.$$

Intracellular cations were calculated according to the method of BOURKE & TOWER (1966). The concentration of Na^+ and K^+ in incubation medium was 140 mM and 5 mM respectively, and the dry weight averaged 17%. The increased wet weight was 9.7 ± 0.6% for control, and ranged from 10.4% to 34.7% for experimentals.

The lactic acid assay consisted of an aliquot of 0.25 ml of the incubation medium, 13.0 units of lactate dehydrogenase, 1.3 mg of β-NAD (Sigma, St. Louis, MO) in glycine-hydrazine buffer, pH 9.2 with a final volume of 1.5 ml.

Protein and adenosine 5′-triphosphate (ATP) in cortical slices were measured separately after 90 min incubation. Cortical slices were homogenized with 1 ml of 6% trichloroacetic acid, 10 μl of homogenate was used for protein assay according to the method of LOWRY *et al.* (1951) with a slight modification. 0.1 ml of 0.15% sodium deoxycholate was first added into 1 ml cortical slice homogenates in order to increase the solubility of the membraneous protein. The protein concentration was 145.1 ± 6.1(5) mg/g of wet tissue for control, and were 153.3 ± 7.4(7), 137.8 ± 7.2(8) and 150.5 ± 5.2(6) for L-glutamate, kainic and DL-homocysteic acids-treated slices respectively. 0.5 ml of each aliquot was used to measure the ATP in the presence of phosphoglycerate phosphokinase, glyceraldehyde phosphate dehydrogenase and NADH (all of the above enzymes and chemicals were obtained from Sigma, St. Louis, MO) according to the method of ADAMS (1963).

EXPERIMENTAL RESULTS

Effects of neurotransmitter amino acids on the induction of brain swelling in vitro

Table 1 shows that control rat brain cortical slices gained a 10% wet wt increase and a 1.9% swelling after incubation in Krebs–Ringer buffer for 90 min. The tissue Na^+/K^+ ratio was approximately 0.5. The addition of α-amino-isobutyric acid (AIBA), a non-metabolized amino acid, or L-taurine, glycine, GABA, the putative inhibitory neurotransmitter in the CNS, at concentrations of 1 mM, had no effect on cortical swelling (inulin space), lactate production, or intracellular Na^+ or K^+ content. The tissue Na^+/K^+ ratio remained about 0.5. L-glutamate and L-aspartate, the putative excitatory neurotransmitter amino acids, enhanced both the wet weight and brain swelling by twofold with a concomitant inulin space decrease. These data indicate that a specific intracellular swelling was induced by these dicarboxylic amino acids. Intracellular Na^+ was increased 34% and 60% and intracellular K^+ was decreased 25% and 41% by L-glutamate and L-aspartate respectively. The tissue Na^+/K^+ ratio was 0.9 for L-glutamate and 1.3 for L-aspartate, suggesting the latter had a greater effect on cation transport in cortical slices. Both the dicarboxylic amino acids stimulated the lactate production by 24–39%.

At an incubation concentration of 10 mM, both L-glutamate and L-aspartate increased brain swelling by threefold with a concomitant decrease in extracellular space. The intracellular cation ratios Na^+/K^+ were dramatically increased to 2.1 and 13.1 by L-glutamate and L-aspartate respectively. However,

TABLE 1. EFFECTS OF NEUROTRANSMITTER AMINO ACIDS ON THE INDUCTION OF BRAIN SWELLING *in vitro*

Incubation medium	mM	Wet weight increase (%)	Swelling (%)	Inulin space (%)	Na^+ (mEq/kg i.w.w.)	K^+ (mEq/kg i.w.w.)	Na^+/K^+	Lactate production (nmol/kg d.w.)
Control	1	9.7 ± 0.6 (20)	1.9 ± 0.1 (20)	46.2 ± 0.8 (20)	72.7 ± 2.9 (20)	146.1 ± 4.2 (20)	0.5	231.7 ± 5.6 (20)
α-Amino isobutyric acid	1	10.4 ± 1.1 (7)	1.9 ± 0.3 (7)	49.6 ± 4.0 (3)	73.5 ± 5.1 (7)	144.7 ± 11.5 (7)	0.5	217.2 ± 15.6 (7)
L-Taurine	1	10.7 ± 1.4 (6)	1.9 ± 0.2 (6)	49.0 ± 4.9 (3)	83.6 ± 5.9 (6)	146.5 ± 15.3 (6)	0.6	271.7 ± 48.9 (6)
Glycine	1	10.9 ± 1.2 (7)	2.0 ± 0.3 (7)	47.8 ± 0.8 (3)	74.6 ± 9.4 (5)	151.6 ± 5.8 (5)	0.5	210.9 ± 12.4 (5)
GABA	1	12.8 ± 1.7 (7)	2.1 ± 0.2 (7)	49.6 ± 5.2 (3)	68.7 ± 8.8 (7)	142.1 ± 5.0 (7)	0.5	245.8 ± 13.5 (6)
L-Glutamic acid	1	20.0 ± 1.3 (9)*	3.5 ± 0.2 (9)*	39.0 ± 1.1 (5)*	97.4 ± 4.6 (7)*	109.7 ± 3.7 (6)*	0.9	322.0 ± 28.3 (5)*
	10	34.7 ± 1.9 (9)*	5.3 ± 0.3 (12)*	31.7 ± 2.6 (5)*	150.4 ± 9.5 (7)*	72.7 ± 7.1 (5)*	2.1	325.1 ± 37.9 (9)‡
L-Aspartic acid	1	27.1 ± 4.3 (7)*	4.3 ± 0.4 (7)*	35.9 ± 2.1 (5)*	116.5 ± 6.1 (5)*	86.6 ± 5.3 (5)*	1.3	287.9 ± 14.6 (5)*
	10	33.3 ± 1.2 (9)*	5.3 ± 0.1 (12)*	39.3 ± 2.2 (6)†	190.1 ± 10.5 (7)*	14.5 ± 1.3 (7)*	13.1	185.9 ± 13.4 (9)†

Control and experimental brain tissues Mean Values ± S.E.M. are given. The number of determinations are given in parentheses. Each determination represents a single cortical slice obtained from each hemisphere. *$P < 0.001$, †$P < 0.01$, ‡$P < 0.05$, using Student's *t*-test for statistical analysis. i.w.w. = initial wet wt; d.w. = dry wt.

TABLE 2. EFFECTS OF STRUCTURAL ANALOGS OF EXCITATORY NEUROTRANSMITTER AMINO ACIDS ON BRAIN SWELLING INDUCTION *in vitro*

Analogs	(n)	Wet weight increase (%)	Swelling (%)	Inulin space (%)	Na^+ (mEq/kg i.w.w.)	K^+ (mEq/kg i.w.w.)	Na^+/K^+	Lactate Production (mmol/kg d.w.)
Control	20	9.7 ± 0.6	1.9 ± 0.1	46.2 ± 0.8	72.7 ± 2.9	146.1 ± 4.2	0.5	231.7 ± 5.6
				L-Glutamic acid analogs:				
L-Pyroglutamic acid	4	10.6 ± 2.8	1.6 ± 0.4	47.6 ± 3.7	82.7 ± 15.7	146.7 ± 7.5	0.6	232.2 ± 27.9
Glutaric acid	6	10.8 ± 1.2	2.0 ± 0.2	48.7 ± 0.4	101.5 ± 21.2‡	127.0 ± 13.5	0.8	233.4 ± 23.9
D-Glutamic acid	5	23.4 ± 1.4*	3.9 ± 0.2*	34.1 ± 3.7*	121.7 ± 10.4*	95.8 ± 7.5*	1.3	287.9 ± 12.1*
N-methyl-DL-glutamic acid	8	33.5 ± 1.7*	5.1 ± 0.2*	39.0 ± 3.6	133.9 ± 4.3*	88.5 ± 2.6*	1.5	176.0 ± 9.7*
Kainic acid	9	36.4 ± 0.6*	5.6 ± 0.1*	31.9 ± 1.9*	127.8 ± 5.8*	56.6 ± 2.6*	2.3	152.1 ± 4.9*
DL-Homocysteic acid	8	37.6 ± 1.9*	5.6 ± 0.1*	36.6 ± 1.9*	146.6 ± 4.5*	88.1 ± 6.9*	1.7	193.7 ± 11.1*
				L-Aspartic acid analogs:				
L-cysteine	5	9.2 ± 1.1	1.8 ± 0.2	46.1 ± 4.1	95.5 ± 4.8*	140.5 ± 4.4	0.7	250.7 ± 9.6
N-methyl-DL-aspartic acid	5	18.9 ± 1.9*	3.5 ± 0.2*	34.2 ± 1.1*	102.7 ± 6.9*	108.6 ± 9.0*	0.9	263.4 ± 14.5‡
α-Methyl-DL-aspartic acid	5	19.6 ± 1.5*	3.3 ± 0.2*	35.8 ± 4.2*	125.6 ± 21.6*	94.8 ± 3.9*	1.3	285.4 ± 8.7*
D-Aspartic acid	5	26.4 ± 2.9*	4.2 ± 0.3*	34.8 ± 3.3*	107.4 ± 8.9*	94.2 ± 8.6*	1.1	264.4 ± 9.6‡
L-Cysteic acid	5	23.9 ± 1.7*	4.1 ± 0.2*	32.5 ± 5.9*	105.1 ± 3.1*	70.0 ± 5.5*	1.5	322.3 ± 13.7*

Control and experiments brain tissues Mean Values ± S.E.M. are given. (n) = number of determinations. Each determination represents a single cortical slice obtained from each hemisphere. *$P < 0.001$, †$P < 0.01$, ‡$P < 0.05$, using Student's *t*-test for statistical analysis. The concentration of structural analogs of dicarboxylic amino acids was 1 mM. i.w.w. = initial wet weight; dw = dry weight.

L-aspartate inhibited lactate formation by 20% whereas L-glutamate stimulated the lactate production by 40%.

Effects of structural analogs or isomers of excitatory neurotransmitter amino acids on the induction of brain swelling in vitro

Certain structural analogs, derivatives and isomers of the dicarboxylic amino acids have been identified as potent excitants (CURTIS & WATKINS, 1963). Their effects on brain swelling induction were studied to elucidate the relationship between the swelling-induced actions and the structural and molecular specificity of the dicarboxylic amino acids. Table 2 shows the effects of the structural analogs or isomers of L-glutamic acid and L-aspartic acid on brain swelling induction. The L-glutamic acid analogs, DL-homocysteic acid, kainic acid, and *N*-methyl-DL-glutamic acid, at a concentration of 1 mM, significantly increased intracellular swelling by approximately 2.5-fold. Intracellular Na^+ was increased by less than twofold and intracellular K^+ was depleted by twofold. The cation ratio of Na^+/K^+ was 1.5 for *N*-methyl-DL-glutamic acid, 2.3 for kainic acid and 1.7 for DL-homocysteic acid. However, lactate production was inhibited by a range of 16–34%. The D-isomer of glutamic acid induced intracellular swelling by one-fold, and the Na^+/K^+ ratio was 1.3, indicating D-glutamic acid was less effective than other analogs studied above. However, D-glutamate, similar to its L-isomer, increased lactate production by 24%. L-pyroglutamic acid and glutaric acid had no effect on intracellular swelling, lactate production and intracellular cations level.

Among the structural analogs or isomers of L-aspartic acid, D-aspartic acid and L-cysteic acid increased the intracellular swelling by twofold, followed by *N*-methyl-DL-aspartic acid and α-methyl-DL-aspartic acid, which increased intracellular swelling by 1.5-fold. The intracellular Na^+ was stimulated 41–76% and intracellular K^+ was depleted 34–108% by these compounds. The Na^+/K^+ ratio ranged from 1.0 for *N*-methyl-DL-aspartic acid to 1.5 for cysteic acid. Unlike the structural analogs of L-glutamate, the analogs of L-aspartate increased lactate production 14–39%. Although Na^+ was increased in cortical slices, L-cysteine had no effect on intracellular swelling, lactate production and K^+ content.

Time-course and dose-response kinetics of L-*glutamate and its analogs on brain edema formation* in vitro

Intracellular swelling was significantly increased by L-glutamate and by its analogs after 15 min of incubation. Tissue swelling induced by these amino acids was time-dependent up to 60 min of incubation (Fig. 1A). DL-Homocysteic acid and kainic acid had a much greater effect on cortical swelling than L-glutamate and D-glutamate throughout the time course

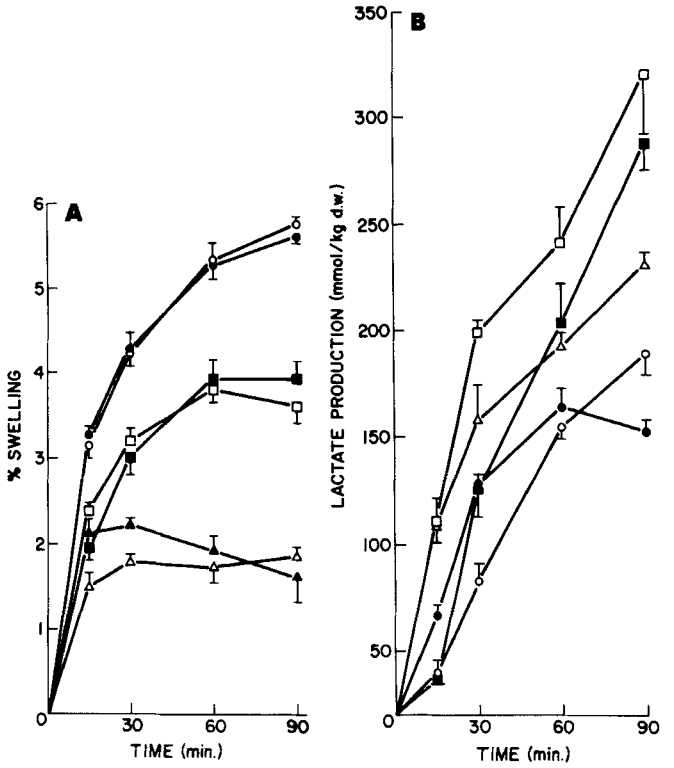

FIG. 1. Time-course studies of the effects of L-glutamic acid and its structural analogs and isomers on cortical swelling and lactate production. —△— Krebs–Ringer buffer; —▲— L-pyroglutamic acid; —□— L-glutamic acid; —■— D-glutamic acid; —○— kainic acid; —●— DL-homocysteic acid. Each point is the mean of 3 to 5 cortical slices ± S.E.M. The amino acid concentration is 1 mM.

studies. L-Pyroglutamate had only a slight effect after early times of incubation. Lactate production was not stimulated at 15 min by L-glutamate and was unchanged by incubation up to 60 min by D-glutamate. Both DL-homocysteic acid and kainic acid inhibited lactate production throughout the time course studies (Fig. 1B). The tremendous increases in tissue swelling with the decreases in lactate production produced by both DL-homocysteic acid and kainic acid, suggest that these amino acid analogs had a toxic effect on cellular metabolism.

Since the amino acid concentration used for these studies was 1 mM, the relationship between lower concentrations and the induction of brain edema by L-glutamate, DL-homocysteic acid and kainic acid required further investigation. Figure 2A shows that both DL-homocysteic acid and kainic acid caused tissue swelling at 10 μM. A profound increase in tissue swelling was observed when the amino acid concentration was raised to 50 μM. L-Glutamate had a similar dose–response pattern on tissue swelling, although with a lesser effect than its analogs. A biphasic effect was seen in lactate production with the incubation of different concentration of DL-homocysteic acid and kainic acid (Fig. 2B). Low doses of these amino acids stimulated lactate production, whereas the high doses had an opposite effect. Lactate production induced by L-glutamate was higher with both low and high dose levels than the control rate.

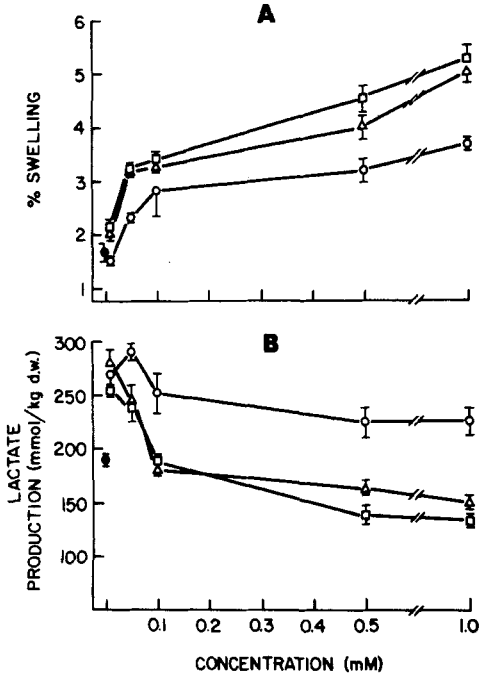

FIG. 2. Dose–response studies of the effects of L-glutamic acid, kainic acid and DL-homocysteic acid on cortical swelling and lactate production. —●— Krebs–Ringer buffer; —○— L-glutamate; —△— kainic acid; —□— DL-homocysteic acid. Each point represents a mean of 3 to 5 cortical slices ± S.E.M. The incubation time is 90 min.

TABLE 3. EFFECTS OF L-GLUTAMATE, KAINIC ACID AND DL-HOMOCYSTEIC ACID ON ATP LEVELS IN CORTICAL SLICES

	n	ATP (nmol.mg protein^{-1})
Control	5	20.1 ± 1.8
L-Glutamate	5	16.3 ± 1.1
Kainic acid	5	13.0 ± 1.5*
DL-Homocysteic acid	4	12.8 ± 1.3*

Control and experimental brain tissues mean values ± S.E.M. are given. (n) = number of cortical slices. *$P < 0.02$, using Student's t-test.

Effects of L-glutamate, kainic acid and DL-homocysteic acid on total ATP levels in cortical slices

Table 3 shows the effect of L-glutamate, kainic acid and DL-homocysteic acid at 1 mM on ATP levels in cortical slices after 90 min incubation. The control ATP level was 20.1 ± 1.8(5) nmoles/mg protein. Both kainic acid and DL-homocysteic acid depleted the high energy adenosine triphosphate by 36%, whereas L-glutamate had no effect.

DISCUSSION

The excitatory neurotransmitter dicarboxylic amino acids and their structural analogs and isomers had a profound effect on brain swelling induction. This intracellular swelling was characterized by increased cellular H_2O, intracellular Na^+ and lactate production, with a concomitant decrease in intracellular K^+ and inulin space. These features are common to other forms of cellular edema associated with a fall in ATP and energy charge (FISHMAN *et al.*, 1977*a*; CHAN & FISHMAN, 1978). The induction was apparently specific to excitatory amino acids, since the putative inhibitory neurotransmitters, such as GABA, glycine and taurine as well as α-amino-isobutyric acid (AIBA), a non-metabolized amino acid, had no effect. It has been demonstrated by BRADFORD & MCILWAIN (1966) that L-glutamate, L-aspartate and several structural analogs in concentrations ranging from 1 to 30 mM, can depolarize the cellular membrane and cause increased influx of Na^+ and decreased inulin space in guinea pig cortical slices. Glutamate increased by 5 fold the tissues' permeability to sodium relative to that to potassium. The additional sodium entering the tissue was considered to accelerate its Na^+, K^+-dependent ATPase and to lead to a fall of the nucleotide phosphates. Our data indicate that kainic acid and DL-homocysteic acid increased both intracellular swelling and intracellular Na^+ and decreased nucleotide triphosphates. Our studies have further demonstrated a significant increment in Na^+/K^+ ratio induced by dicarboxylic amino acids, but the total cation content in rat cortical slices remained constant, suggesting either the ATPase system may be inhibited or a transport system other

than ATPase is involved. These questions might be answered in determining the Na^+, K^+-dependent ATPase activities in the swollen cortical slices. OKAMOTO & QUASTEL (1970) further demonstrated that L-glutamate and D-glutamate at 10 mM concentrations increased water uptake and Na^+ influx and decreased ATP levels in rat brain cortex. Low concentration (0.5 mM) of L-glutamate did not affect cellular energy levels, although water uptake and Na^+ influx were significantly increased. Our studies of dose response on lactate production for L-glutamate, kainic acid and DL-homocysteic acid showed a biphasic response which was independent of the increases in tissue swelling. This indicates that a low concentration of excitants may accelerate cellular glycolysis and thus enable maintenance of a constant level of ATP whereas a high concentration of the amino acid analogs impairs cellular glycolysis which leads to a fall of lactate and energy level.

It has been shown by COX *et al.* (1977) that oxygen uptake was increased by L-glutamate, L-homocysteic acids and their respective isomers, and by kainic acid at a 5–10 mM concentration. Only L-glutamate and D-glutamate increased lactate production after 10 min incubation, whereas homocysteic acid and kainic acid had no effect. At a concentration of 1 mM, kainic acid increased oxygen uptake in cortical slices (COX *et al.*, 1977) but failed to induce intracellular swelling in strial slices (BIZIERE & COYLE, 1978). Unlike these findings, our time course studies show that 1 mM of kainic acid and DL-homocysteic acid increased tissue swelling and decreased lactate production with 15–90 min incubation. Tissue swelling was increased 100% and lactate production was depleted by a similar order of magnitude. These data indicate a direct toxic effect of kainic acid and DL-homocysteic acid on brain tissue, as observed by OLNEY *et al.* (1971, 1974). Whether the discrepancy in kainic acid-induced tissue swelling between cortical and striatal slices may be due to their differences in structure requires further elucidation.

CURTIS & WATKINS (1963) have shown that dicarboxylic amino acids and their structural analogs with the property of acidic groups exhibited a potent neuroexcitatory effect on mammalian neurones. The molecular specificity of L-glutamate underlying its physiological action has been defined by VAN GELDER (1971). Our present studies have demonstrated that among L-glutamic acid analogs, DL-homocysteic acid, kainic acid, *N*-methyl-DL-glutamic acid and D-glutamic had a potent excitatory effect on cortex and caused a profound increase in intracellular swelling, whereas L-pyroglutamate and glutaric acid had no effect. Among the analogs of L-aspartic acid, L-cysteic acid, D-aspartic acid, α-methyl-DL-aspartic acid and *N*-methyl-DL-aspartic acid were effective whereas L-cysteine had no effect. These data indicate that the degree of brain swelling induced by the structural analogs and isomers of the dicarboxylic amino acids depends on the molecular configuration of their amino groups and two anionic groups. The differential effects of the dicarboxylic amino acids on neurones and glia and on various subcellular compartments, requires further elucidation. Present studies suggest that excitatory neurotransmitters play an important role in the induction of brain swelling induction. We speculate that their release in brain *in situ* under various pathophysiological conditions may contribute to the formation of cellular swelling *in vivo*.

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