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Excitatory Acidic Amino Acids and the Cation Content and Sodium Ion Flux of Isolated Tissues from the Brain

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1. Samples from the neocortex and piriform cortex of guinea pigs and rats were incubated in inulin-containing glucose–saline. Their intracellular (non-inulin) space contained 19–27 μequiv. of Na^+/g. of original tissue. These values were stable between 30 and 100 min. after incubation commenced, but addition of $^{22}NaCl$ to the neocortical samples showed them to be associated with a flux of 400 μequiv. of Na^+/g. of tissue/hr. 2. Addition of 0·5–10 mM-L-glutamic acid or 0·1 mM-*N*-methyl-DL-aspartic acid rapidly increased the tissue's Na^+ content; *N*-acetyl-DL-aspartic acid was without action. 3. During the first 1–1·5 min. after the addition of L-glutamic acid to neocortical samples their Na^+ content increased at 600 μequiv./g. of tissue/hr., and the rate of $^{22}Na^+$ influx corresponded to 1230 μequiv. of Na^+/g./hr. These rates were calculated to be sufficiently rapid to account for loss of the tissue's normal membrane potential within 1–2 sec. of the addition of the acid. 4. In addition, a rapid but more limited loss of K^+ took place after the addition of L-glutamic acid or the methylaspartic acid; on continued incubation tissue K^+ content increased, as also did the intracellular volume of the tissue, from its original 670 μl./g. to 1100 μl./g. 5. Interpretation of these and of associated changes is offered in terms that involve a cation pump and the permeability changes associated with the nerve impulse.

Small quantities of L-glutamic acid applied to the mammalian brain *in vivo* accelerate the normally moderate rate of cell firing (Hayashi, 1956; Krnjević & Phillis, 1963). Free L-glutamic acid is a major cerebral constituent and metabolite (see McIlwain, 1966) undergoing many of the interconversions common to the metabolic roles played by the acid in most organs of the body, but in addition it is decarboxylated to γ-aminobutyric acid in a reaction that can proceed at about 20 μmoles/g. of tissue/hr. L-Glutamic acid and γ-aminobutyric acid are sufficiently potent in accelerating and retarding cell firing to merit consideration as excitatory and inhibitory transmitting agents. The mode of action proposed for L-glutamic acid is that it causes a diminution of membrane potential, and hence an increase in cell firing, by entry of the Na^+ cation to the negatively charged cell interior (Curtis & Watkins, 1960, 1965; Eccles, 1964). A direct demonstration of the Na^+ entry was first given with isolated cerebral tissues, which indeed showed, by direct analysis, an increase in Na^+ content on the addition of L-glutamic acid (Bradford & McIlwain, 1966).

To gain further information on the relationships between these properties of L-glutamic acid, the following observations have now been made with isolated tissues from rat and guinea-pig brain. (1) Entry of $^{22}Na^+$ to the tissue in the presence and in the absence of L-glutamate has been measured. (2) Net changes in Na^+ and K^+ have been measured at a series of intervals after the addition of L-glutamate, including periods both shorter and longer than those previously examined. A reason for including longer time-intervals was that Terner, Eggleston & Krebs (1950) found that after 1 hr. incubation *in vitro* L-glutamate led to an increased content of K^+ in cerebral tissues. (3) Further, comparable measurements have been made after the addition of *N*-methyl-DL-aspartic acid, an isomer of glutamic acid and especially potent in inducing cell firing (Curtis & Watkins, 1960), and after the addition of *N*-acetyl-DL-aspartic acid, which occurs in the mammalian brain in quantities comparable with those of free glutamic acid. (4) Also, as different parts of the brain can differ notably in chemical characteristics, samples from the piriform lobes have been compared with those from neocortex. The piriform lobes were chosen because they are important and quite extensive in rodents, and have been the subject of recent electrophysiological studies *in vitro* (Yamamoto & McIlwain,

1966*a*,*b*; Campbell, McIlwain, Richards & Somerville, 1967).

METHODS

Tissues were prepared and incubated as described by McIlwain & Rodnight (1962) unless otherwise specified. Guinea pigs were stunned by a blow to the back of the neck and exsanguinated by cutting the carotid arteries. The brain was rapidly removed and placed in a Petri dish, which was kept humid with a piece of filter paper soaked in the incubating fluid. The brain was split longitudinally with a sharp spatula. The ventral portion of each hemisphere was obtained by cutting along the rhinal fissure with a scalpel. This slab of tissue was placed ventral surface upwards on a cutting block, and a slice of the cortex of the piriform lobe, including the lateral olfactory tract (see Yamamoto & McIlwain, 1966*a*,*b*) was obtained with a bow-cutter and recessed glass guide, used dry and without incubating fluid. The remaining portion of the cerebral hemisphere was divided into approximately equal parts, which carried the anterior and posterior neocortex. One slice, carrying the original outer cortical surface, was obtained from each of these slabs in the same manner as described above. All slices, approximately 350μ thick, were transferred immediately after being cut to a bent wire rider, weighed to the nearest milligram on a torsion balance and placed in a 30ml. beaker containing 4·85ml. of a glucose–bicarbonate incubating fluid at 37·5°. Through the incubating fluid a gas mixture of O_2+CO_2 (95:5) was constantly bubbled; the fluid contained (mM, final concns.): NaCl, 124; KCl, 5; KH_2PO_4, 1·24; $MgSO_4$, 1·3; $NaHCO_3$, 26; $CaCl_2$, 0·75; glucose, 10; and also 1% of inulin. The fluid was equilibrated with a gas mixture of O_2+CO_2 (95:5) before use.

After 40min. incubation, additions were made of 0·1ml. of either a neutralized amino acid solution or an equimolar solution of NaCl and, following this within 15sec. when specified, an addition of 0·05ml. of 0·154M-^{22}NaCl was delivered by means of a glass syringe to each. When present, the final concentration of radioactivity was approximately 1·5μc/ml. Zero time was taken as the time at which ^{22}NaCl was added. At various times after the addition of ^{22}NaCl, each slice was picked up from the beaker on a bent wire rider, care being taken to avoid contact of the slice with the side of the beaker. The slice was then rapidly drained on a clean glass surface, reweighed to the nearest milligram and ground in 4ml. of 6% (w/v) trichloroacetic acid in a test-tube homogenizer. The suspensions were kept at room temperature for 30min. and were then centrifuged at 800*g* for 15min. The supernatants were collected in volumetric flasks and each was combined with a second supernatant obtained by resuspending the residue in 4ml. of glass-distilled water and recentrifuging. The tissue extracts were then diluted to 25ml. with water. A sample (0·1ml.) of each incubation fluid was added to 8ml. of 6% (w/v) trichloroacetic acid and diluted to 50ml. for analysis.

Determinations. Na^+ and K^+ were measured in each tissue extract and in two or more of the incubation media by flame photometry (model A; Evans Electroselenium Ltd., Halstead, Essex) with appropriate filters. Standards for the tissue extract contained Na^+ and K^+ in the molar ratio 2:1, and 24:1 for the incubation media. All glassware was treated with HNO_3 before use, as described by Keesey, Wallgren & McIlwain (1965).

Inulin was determined by the method of Varon & McIlwain (1961) with 5ml. samples of tissue extract or medium.

For the determination of radioactivity duplicate 0·2ml. samples of tissue extracts or of incubating fluids were plated directly on to stainless-steel planchets, and their radioactivity was measured in an open-window gas-flow Geiger counter (Nuclear–Chicago Corp.). Samples of media to which no radioactivity had been added were plated and counted to give the background radioactivity. All samples had at least 20 times the background radioactivity, and counting was terminated after 1000 counts had been obtained.

Expression of results. Quantities of ions were first expressed as μequiv./g. initial wt. of tissue. Inulin space expressed as μl./g. initial wt. of tissue was calculated as:

$$\text{Inulin space } (\mu\text{l./g.}) = \frac{\text{mg. of inulin/g. of tissue}}{\text{mg. of inulin/}\mu\text{l. of medium}}$$

A $^{22}Na^+$ space expressed as μl./g. initial wt. of tissue was obtained in a similar manner:

$$^{22}Na^+ \text{ space } (\mu\text{l./g.}) = \frac{\text{counts/min./g. of tissue}}{\text{counts/min./}\mu\text{l. of medium}}$$

The size of the $^{22}Na^+$ space relative to the inulin space was obtained as the ratio $^{22}Na^+$ space/inulin space. The influx of Na^+ into the slice was expressed as μequiv./g. initial wt. of tissue, and was calculated by multiplying the $^{22}Na^+$ space (μl./g.) by the Na^+ content of the incubating fluid (μequiv./μl.). Similarly, the Na^+ content of the inulin space was determined by multiplying the inulin space (μl./g.) by the Na^+ content of the fluid (μequiv./μl.). The quantity of Na^+ exchanging in the non-inulin space was then calculated by subtracting the content of Na^+ in the inulin space from the quantity of Na^+ exchanging with the whole slice, and expressed as μequiv./g. initial wt. of tissue. The non-inulin space was obtained by subtracting the inulin space from the total volume of the tissue after incubation, the volume of tissue being assumed to be 1000μl./g. initial wt. The assumptions on which these calculations are made have been discussed by Varon & McIlwain (1961), Keesey *et al.* (1965) and Keesey & Wallgren (1965). The significance of the differences between samples means were obtained by Student's *t* test (two-tailed).

Materials. Chemicals were laboratory reagents of the highest available chemical purity. Sodium L-glutamate monohydrate was dissolved in glass-distilled water before use. *N*-Acetyl-DL-aspartic acid and *N*-methyl-DL-aspartic acid monohydrate (kindly supplied by Dr J. C. Watkins) were first dissolved in a minimal amount of 0·1N-NaOH and then diluted to appropriate concentrations with glass-distilled water. The pH of the amino acid solutions was approximately 7·5, determined by a microcapillator with phenol red as indicator. The ^{22}NaCl was supplied as an iso-osmotic solution by The Radiochemical Centre, Amersham, Bucks.

RESULTS

A specific pattern of change, described first below in relation to neocortical tissues, resulted from the addition of L-glutamate to guinea-pig neocortex and piriform cortex and also to rat neocortex.

Table 1. *Effects of* L-*glutamate and* N-*substituted aspartates on the composition of cerebral cortical tissues*

Tissues were incubated for 30 min. in the glucose–bicarbonate medium containing 1% (w/v) inulin (see the text) and in equilibrium with O_2+CO_2 (95:5). After additions of amino acids or an equivalent amount of NaCl, slices were removed at the intervals indicated and ground in 6% (w/v) trichloroacetic acid for analysis. Results obtained with slices receiving NaCl are pooled for each tissue employed. The symbols appearing after quoted means ±S.D. indicate the levels of significance as determined by Student's *t* test (two-tailed) as follows: *, 0·05; †, 0·02; ‡, 0·01; §, 0·005; ‖, 0·001. The tissue content of ions is given as μequiv./g. initial wt. of tissue; the concn. of ions (mM) refers to μequiv./g. non-inulin space of the incubated tissue.

Amino acid added and its concn. (mM)	Time after addition (min.)	No. of samples	Total Na^+ (μequiv./g.)	Total K^+ (μequiv./g.)	Non-inulin-space Na^+ (μequiv./g.)	Non-inulin-space K^+ (μequiv./g.)	Na^+ of non-inulin space (mM)	K^+ of non-inulin space (mM)	Inulin space (μl./g.)	Non-inulin space (μl./g.)	Increase in weight (%)
					Guinea-pig neocortex, superficial 350 μ						
None		33	120±9	76±5	25±5	73±5	37±6	110±13	581±55	669±72	25±8
L-Glutamate, 5	0·5	4	128±7	66±2‖	31±9†	62±8‖	48±8‖	100±13	590±63	634±88	22±6
	1	4	126±8	67±4‖	33±5§	64±4§	51±4‖	100±13	567±59	640±54	21±5
	2	4	131±6†	64±4‖	42±2‖	61±4‖	59±2‖	84±3‖	532±39	724±45	25±6
	5	11	136±9‖	61±7‖	59±11‖	59±7‖	72±11‖	72 ±12‖	466±69‖	824±85‖	29±7
	10	4	152±5‖	61±3‖	73±9‖	58±3‖	80±6‖	64±5‖	466±28‖	906±36‖	37±3
	60	4	162±5‖	90±11‖	76±8‖	86±11‖	68±4‖	78±11‖	500±38	1111±42‖	61±5
N-Acetyl-DL-aspartate, 5	5	6	113±6	77±3	24±3	73±3	37±4	114±5	559±36	640±25	20±3
N-Methyl-DL-aspartate, 0·1	2	4	124±7	70±1‡	36±9‖	66±1‡	54±8‖	101±9	576±89	658±75	23±4
	5	4	130±11*	62±2‖	49±12‖	60±2‖	64±13‖	78±7‖	532±29	768±44†	30±6
					Guinea-pig piriform lobe, superficial 350 μ						
None		8	102±8	70±4	19±6	67±3	32±7	113±17	496±50	600±74	10±4
L-Glutamate, 5	5	4	115±3‡	63±5†	38±4‖	60±5†	54±9‡	86±4†	468±50	699±65*	17±7*
					Rat neocortex at 0–350 and 350–700 μ from surface						
None		4	137±14	66±4	27±5	61±4	41±8	94±5	729±76	656±49	39±6
L-Glutamate, 5	5	4	152±8	60±5	57±19*	56±5	70±14‡	72±15*	626±95	797±99	42±6

Net changes in neocortical cation and water content. L-Glutamate added to tissues being incubated in the glucose–bicarbonate medium caused a large and rapid redistribution of Na^+ and K^+ in the tissue samples (Table 1). In the absence of such addition, the composition of the tissues was stable between 40 and 100 min. of incubation, but when the medium was made 5 mM in L-glutamate, tissue K^+ fell and Na^+ increased. When the ion content was expressed in terms of fluid spaces accessible or not accessible to inulin (see the Methods section), the changes were large 30 sec. after the addition. Compared with control tissues, those with added L-glutamate gave P 0·005 at 1 min. Between zero and 1 min. the net rate for Na^+ entry was 600 μequiv./g. of original tissue/hr., and the rate for K^+ loss was 660 μequiv./g. of tissue/hr. Between 10 and 60 min. after addition an opposite movement of K^+ occurred so that finally both Na^+ and K^+ contents of the non-inulin space were higher than in control slices.

Fluid content and distribution, measured by total weight of the samples and by inulin content, remained close to their original values during the initial few minutes after L-glutamate addition (Table 1). At this time the movements of Na^+ and K^+ were large but were reciprocal. At 5 min. after the addition of glutamate, marked diminution occurred in the inulin space of the samples, and this was accompanied by an increase in the non-inulin space, without tissue swelling. The non-inulin space of the slices continued to increase at 10 and 60 min. after the addition of L-glutamate, but this further increase was paralleled by a concomitant increase in tissue swelling.

The net result of these movements was to give an almost constant value for the sum of tissue Na^+ and K^+ concentrations in the non-inulin space, throughout the 60 min. period. Table 2 shows the change in Na^+ plus K^+ content of the non-inulin space together with the change in water content of the same space. These latter data were obtained by assuming that increases in non-inulin space reflect water uptake, and are expressed as μmoles/g. initial wt. of tissue. The data show a nearly linear relationship between water uptake and content of cations in non-inulin space: approximately 7·2 ml. of water was absorbed for each m-mole of cation. Comparable ratios were found after addition of L-glutamate to tissues from the rat (Table 2).

Of glutamate analogues, *N*-methyl-DL-aspartate, in view of earlier work (Curtis & Watkins, 1960; Bradford & McIlwain, 1966), was examined at a much lower concentration than was L-glutamate, namely at 0·1 mM (Tables 1 and 2). It brought about, 2–5 min. after its addition to guinea-pig neocortex, all the changes described above as resulting from addition of L-glutamate. *N*-Acetyl-DL-aspartate,

Table 2. *Effect of* L-*glutamate and* N-*substituted aspartates on entry of* Na^+ *plus* K^+ *and of water into cortical tissues*

The Na^+ plus K^+ of non-inulin spaces is expressed as content (μequiv./g. initial wt.) and as concn. (mM, being μequiv./g. of non-inulin space of incubated tissue). Incubation procedures were as given in Table 1. The symbols for different levels of significance are as defined in Table 1.

Amino acid added and its concn. (mM)	Time after addition (min.)	No. of samples	Na^+ plus K^+ of non-inulin space (μequiv./g. of initial tissue)	Na^+ plus K^+ of non-inulin space (mM)	Change in non-inulin-space Na^+ plus K^+ (μequiv./g. of initial tissue)	Change in non-inulin space (μl./g.)
Guinea-pig neocortex, superficial 350μ						
None		33	98±7	147±11		
L-Glutamate, 5	0·5	4	94±10	148±7	−4	−35
	1	4	96±5	151±13	−2	−29
	2	4	104±5	143±3	+6	+55
	5	11	118±10‖	144±13	+20	+155
	10	4	131±7‖	144±2	+33	+237
	60	4	162±10‖	146±13	+64	+442
N-Acetyl-DL-aspartate, 5	5	6	97±4	151±7	−1	−29
N-Methyl-DL-aspartate, 0·1	2	4	102±11	156±4	+4	−11
	5	4	108±11†	141±12	+10	+99
Guinea-pig piriform lobe, superficial 350μ						
None		8	86±7	145±16		
L-Glutamate, 5	5	4	98±3*	140±9	+12	+99
Rat neocortex, 0–350 and 350–700μ from surface						
None		4	88±7	134±8		
L-Glutamate, 5	5	4	112±16†	142±4	+24	+141

Table 3. *Effect of* L-*glutamate and* N-*substituted aspartates on influx of* $^{22}Na^+$ *into neocortical tissues*

Tissues were incubated as in Table 1. After 30 min. incubation $^{22}NaCl$ was added, followed within 15 sec. by either an amino acid or an equivalent amount of NaCl. Slices were then removed at the indicated intervals after the addition of $^{22}NaCl$. The symbols after quoted means±S.D. indicate levels of significance, as in Table 1. The influx of $^{22}Na^+$ is expressed as the quantity of Na^+ (μequiv./g. initial wt.) in the slice or in the non-inulin space that has exchanged with the Na^+ of the medium. In addition, the relative size of the $^{22}Na^+$ space (μl./g. initial wt.) is compared with the size of the inulin space (μl./g. initial wt.) as a ratio.

Amino acid added and its concn. (mM)	Time after addition (min.)	No. of samples	Total Na^+ exchanged (μequiv./g.)	Na^+ exchanged in non-inulin space (μequiv./g.)	Ratio of $^{22}Na^+$ space to inulin space
Guinea-pig neocortex, superficial 350μ					
None	0·5	4	90±8	−12±4	0·88±0·03
L-Glutamate, 5	0·5	4	94±9	−1±8*	0·98±0·09
None	1	4	92±10	−5±5	0·95±0·06
L-Glutamate, 5	1	4	109±17	16±10‡	1·16±0·09‡
None	2	6	98±8	2±3	1·02±0·04
N-Methyl-DL-aspartate, 0·1	2	4	102±14	14±3‖	1·17±0·05‖
L-Glutamate, 5	2	4	114±14*	25±10‖	1·31±0·09‖
None	5	8	114±14	22±11	1·20±0·11
N-Acetyl-DL-aspartate, 5	5	6	109±15	19±8	1·21±0·09
N-Methyl-DL-aspartate, 0·1	5	4	121±9	40±5†	1·50±0·06‖
L-Glutamate, 5	5	4	127±4	44±7§	1·53±0·13‖
Rat neocortex, 0–350μ and 350–700μ from surface					
None	5	4	131±19	26±9	1·18±0·05
L-Glutamate, 5	5	4	141±9	46±12*	1·51±0·22*

however, was without action at 5 mM and after experimental periods that allowed large changes with the other two acids.

Influx of $^{22}Na^+$ *ions to neocortical samples.* The influx was first examined (Table 3) by adding $^{22}NaCl$ to fluids in which tissue samples had already been preincubated for 40 min.; unless specified therefore their net composition was stable. Under these

conditions $^{22}Na^+$ entry to the non-inulin space of the samples proceeded initially at a rate corresponding to an entry of 400 μequiv. of Na^+/g. of tissue/hr. This rate diminished as the $^{22}Na^+$ radioactivity increased to a tissue content corresponding to its total non-inulin Na^+; at 5 min. after addition the $^{22}Na^+$ content corresponded to 44 μequiv./g. and the total non-inulin Na^+ to 59 μequiv./g. (Table 1).

When, however, the addition of $^{22}NaCl$ followed a few seconds after the addition of L-glutamate, the entry of $^{22}Na^+$ was much more rapid. During the period between 0·75 and 1·5 min. the entry corresponded to 1230 μequiv. of Na^+/g. of original tissue/hr. Again, this rate diminished subsequently. Influx of Na^+ was increased also by *N*-methyl-DL-aspartate, which again was examined at a much lower concentration than L-glutamate, namely at 0·1 mM (Table 3). At 2 min. after the addition of the *N*-methyl-DL-aspartate the increase in entry of Na^+ was highly significant; the effect of this compound was not examined at a briefer interval, but it was still evident 5 min. after the addition. *N*-Acetyl-DL-aspartate, in contrast, was without effect at 5 mM 5 min. after addition (Table 3).

Cortex from the piriform lobe of the guinea pig. The cortical samples from the piriform lobe were significantly different from neocortical tissues in all the measures of Table 1. The piriform samples were prepared and incubated under the same conditions as those from the neocortex of the same animals, but carried smaller inulin and non-inulin spaces. On incubation they increased in weight by 9·7% in contrast with the 24% found with neocortex (P value of the difference $< 0·001$). The Na^+ and K^+ contents per unit weight were each markedly smaller in the piriform samples. However, when expressed as concentrations of the two ions in the fluid spaces not accessible to inulin, neocortical and piriform samples were nearly equal (Table 1); the sums of Na^+ plus K^+, each non-inulin, were indistinguishable (Table 2).

L-Glutamate, added to piriform tissue under the same conditions as it was added to neocortex, again increased the weight and non-inulin spaces of the samples and increased their Na^+ content. In 5 min., the quantity of Na^+ in the non-inulin space of the piriform samples, expressed in terms of original tissue weight, doubled and its concentration increased by 65%. Also, L-glutamate caused the K^+ content of the piriform samples to fall within 5 min. of its addition (Table 1).

DISCUSSION

Control of permeability to Na^+ ions and its modulation in different functional conditions are central features of the operation of neural systems, though the mechanisms involved are poorly understood. Cerebral tissues as now prepared and examined in isolation clearly exhibit such permeability control. Their normal Na^+ concentration is markedly lower than that of surrounding fluids, but it can increase sharply on electrical stimulation (Keesey *et al.* 1965) or on the addition of L-glutamate (Bradford & McIlwain, 1966). The result of glutamate addition can now be specified in further detail as follows.

(1) The enrichment of the tissue in Na^+ results from increased entry of Na^+, rather than from diminished exit. With L-glutamate, the influx of $^{22}Na^+$ gave initial rates of Na^+ entry of approx. 800 μequiv./g./hr. above those without the added amino acid. As the maximum rate of net entry of Na^+ was 600 μequiv./g./hr., it is unnecessary to suggest that L-glutamate diminishes the exit of Na^+; it may, rather, to some extent increase the exit as well as the entry. Increased movement in both directions is understandable if tissue permeability to Na^+ is increased. A previous calculation (Bradford & McIlwain, 1966) from results on membrane potential suggested that L-glutamate increased fourfold the tissue permeability to Na^+ relative to that to K^+. As L-glutamate does not markedly diminish tissue permeability to K^+ (see below), this calculation and the present findings are compatible.

(2) A relatively small entry of Na^+, if not accompanied by other changes, would suffice to depolarize the tissue cells. The ion equivalence of the membrane potential may be approximately estimated as follows. The area of neuronal outer cell surface in microscopically visible structures in mammalian neocortical samples was estimated by Schadé & Baxter (1960) to be approaching 10^4 cm.^{2}/g., though electron-microscopical examination suggests a possible fivefold increase in this value for finer cell elements (Schadé, Backer & Colon, 1964). Taking the capacity of these membranes as approximately 10 μF/cm.^{2} (see Eccles, 1957), the ion equivalence for a 60 mv charge is about 60 mμequiv./g. for the smaller surface and 300 mμequiv./g. for the larger area. The rate now observed for the initial entry of Na^+ corresponds to 160 mμequiv./g./sec., and the entry could thus depolarize the tissue cells within 2 sec. This is consistent with the observation by Gibson & McIlwain (1965) that depolarization of neocortical cells by L-glutamate commenced within 1–2 sec. of the addition of the compound.

(3) Two types of specialized region are considered to exist at which Na^+ crosses neural membranes. These concern the nerve action potential and the Na^+ pump, and L-glutamate may interact with one or both of these regions. The special mechanisms associated with both are brought into play by

L-glutamic acid (Bradford & McIlwain, 1966). In the series of changes normal to the nerve action potential, the depolarization resulting from Na^+ entry operates a mechanism that increases K^+ permeability (Hodgkin, 1965). After the addition of L-glutamate, some 15% of the tissue K^+ moves (Table 1) from its high intracellular concentration to the extracellular fluids low in K^+. This movement is rapid for about 1 min. and continues at much diminished speed for at least 10 min., in contrast with the period of about 1 msec. occupied by increased K^+ permeability during the nerve action potential. The tissue depolarization after the addition of L-glutamate is also, however, prolonged and indeed continues until extracellular L-glutamate is removed (Gibson & McIlwain, 1965).

(4) The K^+ lost after the addition of L-glutamate is later regained, and L-glutamate also induces a diminution of energy-rich phosphates of the tissues and an increase in their respiration. Previous appraisal of these changes (Bradford & McIlwain, 1966) ascribed them to an increased operation of the Na^+ pump, stimulated by increased intracellular Na^+ concentrations. The present data are consistent with this, and a similar explanation has been discussed for the ion changes and respiratory increase on electrical stimulation of the tissue (McIlwain, 1963).

In considering whether the same restoring (pumping) mechanisms are brought into action by electrical stimulation and by L-glutamate, the Na^+ and K^+ contents of the tissue after exposure to the agents for several minutes are relevant. Such times are presumably adequate for the effects of the agents and of the restoring mechanisms to be displayed. After electrical stimulation of neocortex for 10 min., Na^+ increased by 28 μequiv./g. and K^+ diminished by 22 μequiv./g. (Keesey *et al.* 1965). Exposure to L-glutamate for 10 min. in the present experiments increased Na^+ by 50 μequiv./g. and diminished K^+ by 15 μequiv./g. This greater increase in Na^+ after addition of L-glutamate is associated with increased intracellular fluids and is part of the initial action of L-glutamate itself, which (see above) greatly increased tissue permeability to Na^+ relative to that to K^+. The observations thus do not indicate different restoring processes under the two circumstances.

(5) Much of the interest in the properties of L-glutamate now exhibited lies in their relationship to the excitation that the compound is capable of inducing in several mammalian species *in vivo*. It is therefore encouraging that the glutamate isomer that is active *in vivo*, namely *N*-methyl-DL-aspartate, shares the action of L-glutamate *in vitro*. At a concentration of 0·1 mM, *N*-methyl-DL-aspartate was capable (Table 1) of increasing the non-inulin Na^+ and of diminishing the K^+ of neocortical samples. Like L-glutamate, it also increased the tissues' Na^+ exchange (Table 3). Moreover, the action of L-glutamate was not confined to guinea-pig neocortex but is shown by the results of Tables 1–3 to extend also to the piriform lobe, and to the rat.

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REFERENCES

Bradford, H. F. & McIlwain, H. (1966). *J. Neurochem.* **13**, 1163.

Campbell, J., McIlwain, H., Richards, C. D. & Somerville, A. (1967). *J. Neurochem.* **14**, 937.

Curtis, D. R. & Watkins, J. C. (1960). *J. Neurochem.* **6**, 117.

Curtis, D. R. & Watkins, J. C. (1965). *Pharmacol. Rev.* **17**, 347.

Eccles, J. C. (1957). *The Physiology of Nerve Cells*, p. 21. London: Oxford University Press.

Eccles, J. C. (1964). *The Physiology of Synapses*, p. 71. Berlin: Springer-Verlag.

Gibson, I. M. & McIlwain, H. (1965). *J. Physiol.* **176**, 261.

Hayashi, T. (1956). *Chemical Physiology of Excitation in Muscle and Nerve*, pp. 152–157. Tokyo: Nakayama-Shoten.

Hodgkin, A. L. (1965). *The Conduction of the Nervous Impulse*, pp. 56–70. Liverpool University Press.

Keesey, J. C. & Wallgren, H. (1965). *Biochem. J.* **95**, 301.

Keesey, J. C., Wallgren, H. & McIlwain, H. (1965). *Biochem. J.* **95**, 289.

Krnjević, K. & Phillis, J. W. (1963). *J. Physiol.* **165**, 274.

McIlwain, H. (1963). *Chemical Exploration of the Brain: A Study of Cerebral Excitability and Ion Movement*, pp. 11, 44, 75. Amsterdam: Elsevier Publishing Co.

McIlwain, H. (1966). *Biochemistry and the Central Nervous System*, 3rd ed., pp. 139–149. London: J. and A. Churchill Ltd.

McIlwain, H. & Rodnight, R. (1962). *Practical Neurochemistry*, pp. 112–118. London: J. and A. Churchill Ltd.

Schadé, J. P., Backer, H. von & Colon, E. (1964). In *Progress in Brain Research*, vol. 4, p. 150. Ed. by Purpura, D. P. & Schadé, J. P. Amsterdam: Elsevier Publishing Co.

Schadé, J. P. & Baxter, C. F. (1960). In *Inhibition in the Nervous System*, p. 207. Ed. by Roberts, E. & Baxter, C. F. New York: Pergamon Press Inc.

Terner, C., Eggleston, L. V. & Krebs, H. A. (1950). *Biochem. J.* **47**, 139.

Varon, S. & McIlwain, H. (1961). *J. Neurochem.* **8**, 262.

Yamamoto, C. & McIlwain, H. (1966*a*). *Nature, Lond.*, **120**, 1055.

Yamamoto, C. & McIlwain, H. (1966*b*). *J. Neurochem.* **13**, 1333.