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Excitotoxic Amino Acids: Research Applications and Safety Implications

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It has been demonstrated repeatedly in recent years that glutamate (Glu), a putative excitatory transmitter and the most abundant amino acid in the mammalian central nervous system, has striking neurotoxic properties. Moreover, it is clear from molecular specificity studies that certain structural analogs of Glu mimic both the neuroexcitatory and neurotoxic effects of Glu and have the same orders of potency for their excitatory and toxic activities. For convenience in referring to those structural analogs of Glu having both neuroexcitatory and neurotoxic activity, we have proposed the term "excitotoxic" amino acids. Because these agents, when injected directly into brain, exert toxic activity against dendrosomal portions of the neuron without damaging axons and because some analogs are much more powerful toxins than Glu itself, neurobiologists are finding excitotoxins quite useful as "axon-sparing" lesioning agents. The ability of excitotoxins to penetrate select regions of the endocrine hypothalamus from blood and to interact with neuroendocrine regulatory units makes these agents also valuable as neuroendocrine investigational tools. Here my major purpose will be to review the development of information pertaining to the neurotoxicity and neuroendocrine interactions of excitatory amino acids. Certain aspects of the food safety issue posed by the use of Glu as a food additive will also be addressed briefly.

SYSTEMIC NEUROTOXICITY OF GLUTAMATE

Background

The first hint that Glu might have neurotoxic potential was provided 20 years ago by Lucas and Newhouse (41) in a report that neurons in the inner layers of the retina rapidly degenerate following subcutaneous (s.c.) administration of Glu to infant mice. Evidence more recently developed includes confirmation of the retinal findings in mice (15,56,90), rats (22,26,33), and rabbits (25); and the demonstration in mice (1,3,4,10,11,29,38,39,47,55,57,70,83,84), rats (3,5,10,11,13,21,51,52,54,

106), guinea pigs (72,106), hamsters (37,101), chicks (99), and rhesus monkeys (59,75,79,81) that nerve cells in the developing brain are also destroyed by systemic Glu administration. Although the subcutaneous route of administration was employed in many of the above studies, Glu-induced brain damage has been demonstrated following oral administration of Glu to mice (39,61,70,106), rats (10, 11,106), guinea pigs (106), and monkeys (81). The lowest effective doses for the two routes of administration are not markedly different (39,61,106).

Other toxic manifestations associated with systemic administration of Glu include convulsions in rats (6,32,100), cats (23), and monkeys (81); vomiting in dogs (42,104), monkeys (81), and man (40); and the "Chinese restaurant syndrome" in human adults (34,93,94,97), which involves intensely disagreeable pain and burning sensations about the face, neck, and torso following ingestion of foods heavily seasoned with Glu. A Glu-intolerance syndrome consisting of "shudder" attacks and headaches has been described in human children (95). Whether or how the latter syndromes in humans relate to the neurotoxicity of Glu demonstrable in experimental animals remains undetermined.

General Features of Glu Neurotoxicity

The brain damage resulting from systemically administered Glu is selective (55,61,76) for certain brain regions, which are said to lie "outside" blood-brain barriers and are known collectively (105) as circumventricular organs (CVOs) (Fig. 1). Both neonatal and adult animals are vulnerable to Glu-induced brain damage, but higher systemic doses are required to damage the brain in adulthood (39,55,61,76). This may be due in part to the greater capacity of the adult liver to metabolize Glu; in any event, the same brain regions are preferentially affected at either age (55,61,76). One CVO region, the arcuate nucleus of the hypothalamus (AH) and contiguous median eminence (ME), has received more attention than others, both because of its particular vulnerability to Glu-induced damage and because of associated neuroendocrine disturbances. Two other CVO regions, the subfornical organ (SFO) and area postrema (AP), are as vulnerable as AH-ME to Glu-induced damage. Future research quite likely will reveal Glu to be a useful tool for probing the functions of these rather obscure brain regions.

Glu lesions, whether in the retina (56) or brain (57,59,71,76,81) and regardless of species, involve rapid swelling of neuronal dendrites and cell bodies followed by acute degenerative changes in intracellular organelles and coarse clumping of nuclear chromatin (Figs. 2 and 3). The reaction is very rapid with onset of dendritic swelling being detectable in 15 to 30 min and phagocytosis of the necrotic neuronal cell body beginning as early as 3 hr after s.c. administration of Glu (56,57). Because axons exhibit no degenerative changes in the acute period, whereas signs of a severe toxic reaction are evident in other neuronal compartments, we have termed this a dendrosomatotoxic but axon-sparing type of cytopathology.

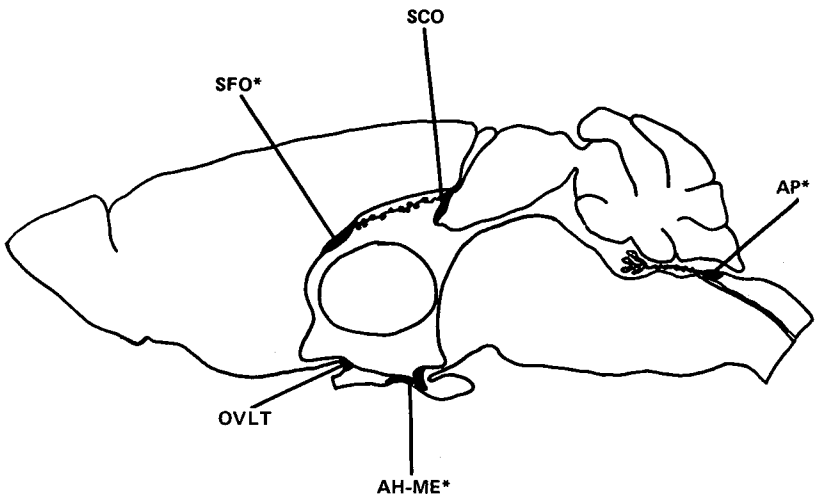


FIG. 1. Diagram of a midsagittal section of rat brain indicates the location of the CVOs, which are special midline periventricular zones that differ from all other brain regions in having fenestrated capillaries that are readily penetrated by various blood-borne substances. Neurons in or near CVOs are subject to damage by systemically administered excitotoxins, whereas other brain regions, even in infancy, are well protected. CVOs include the AP, subcommissural organ (SCO), SFO, organum vasculosum of the lamina terminalis (OVLT), and the AH-ME. Asterisks indicate CVOs most vulnerable to damage by systemically administered excitotoxins.

Molecular Specificity: Clue to Mechanism

In electrophysiological studies, Curtis and colleagues have established that Glu and certain structural analogs of Glu have in common the property of exciting (depolarizing) central mammalian neurons (see chapters by Curtis and Johnston, *this volume*). In a separate series of studies (58,60,65,69,71,73,74,77,80), we administered various Glu analogs s.c. to infant mice and found the excitatory analogs toxic to retinal and hypothalamic neurons, whereas nonexcitatory analogs lacked such toxicity; moreover, it was abundantly clear from these studies that the toxic potency of a given analog parallels its excitatory potency. Coyle et al. (16) in a recent study of the retinotoxic activity of excitatory amino acids, have now corroborated and extended these findings to include additional neuroexcitants not studied by us. Johnston and colleagues (*this volume*) have established that when administered systemically to immature rats, the excitatory amino acids act as convulsants that vary in potency in direct proportion to their known excitatory and toxic activities. Those compounds identified either by us or others as neurotoxic analogs of Glu and that have also been identified as neuroexcitants and convulsants (see chapters by Curtis and Johnston, *this volume*) are depicted in Fig. 4. It is worth emphasizing that these compounds are not merely neurotoxins, but that each appears to exert the same type of neurotoxic action as Glu and that this action, by ultrastructural analysis, is

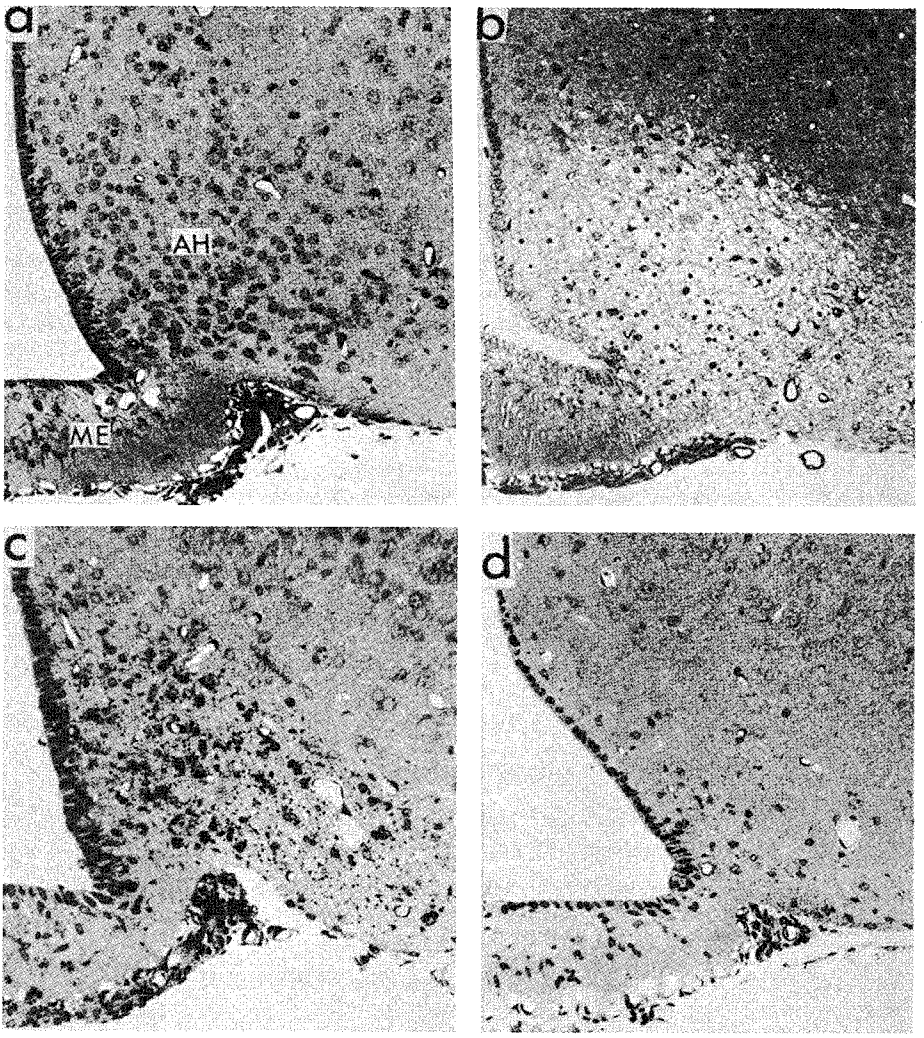


FIG. 2. **a:** The AH and ME regions of the normal 10-day-old mouse hypothalamus. **b:** AH-ME of a 10-day-old mouse 6 hr after a 3 mg/g s.c. dose of Glu. Note the intense edema of cellular components in the AH region, the sharp demarcation of the lesion at the borders of AH with total sparing of other hypothalamic zones and the altered contour of the third ventricle due to AH-ME swelling. **c:** Hypothalamus of an 11-day-old mouse 24 hr after a 3 mg/g s.c. dose of Glu. The AH region is no longer edematous, but is studded with dark-staining dense bodies, which in electron micrographs are recognized as degenerated neurons enclosed within phagocytes. **d:** Hypothalamus of 14-day-old mouse 4 days following a 3 mg/g s.c. dose of Glu. Products of degeneration have disappeared from the AH region, leaving it hypocellular (compare with **a**), and the majority of cells present are nonneuronal. Loss of nerve cell mass is accompanied by compensatory widening of the third ventricle. × 200. (From Olney, ref. 57.)

selective for those regions of the neuron (dendritic and somal) containing the excitatory receptors through which the depolarizing effects of Glu are mediated. In view of these correlations and of the parallel orders of potency for the excitatory and toxic actions of these compounds we have proposed the excitotoxic concept—that a depolarizing mechanism underlies both their excitatory and neurotoxic activities. This concept has been developed and discussed in greater detail elsewhere (62).

MICROINJECTION OF EXCITOTOXINS INTO BRAIN

Cysteine-S-Sulfonic Acid

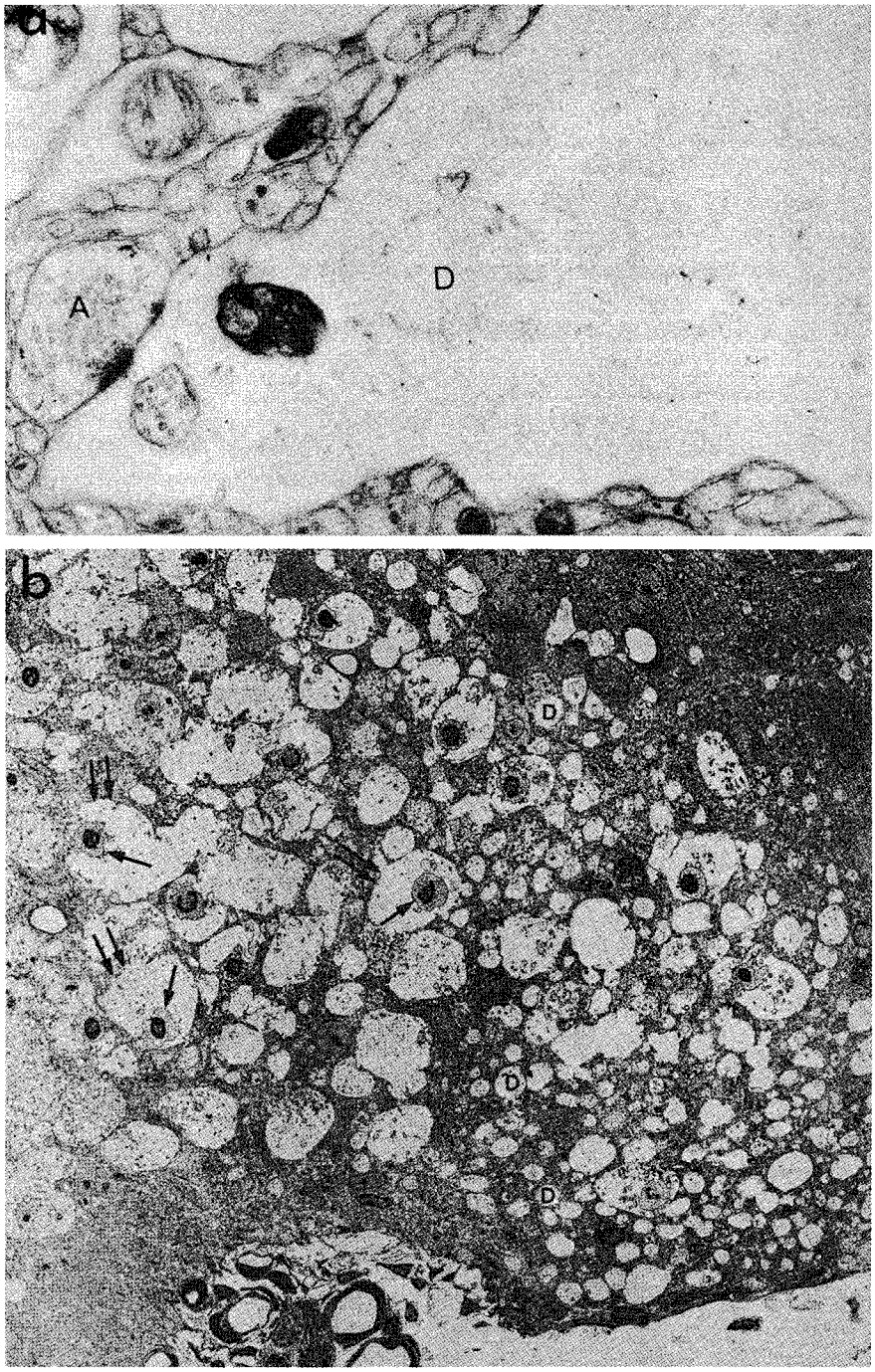
The first Glu analog evaluated by direct microinjection into brain was cysteine-S-sulfonic acid (CSS), an abnormal metabolite associated with the neurodegenerative metabolic disease, sulfite oxidase deficiency. Because CSS resembles the potent excitotoxin, homocysteic acid (HCA) in molecular structure (Fig. 4) and was known to have neuroexcitatory activity, we evaluated its neurotoxic potential following both subcutaneous administration to immature rats and direct microinjection into the brain of the adult rat (73). The latter approach was employed to provide evidence that if CSS were to accumulate in brain, as might occur in cysteine oxidase deficiency, it could have toxic consequences for central neurons. CSS reproduced the Glu-type lesion (dendrosomatotoxic, but axon-sparing) following either subcutaneous administration or direct injection into the diencephalon (62,73).

Homocysteic, N-Methyl Aspartic, and Kainic Acids

As an extension of the CSS experiments, we microinjected three of the more potent Glu analogs—DL-homocysteic acid (HCA), N-methyl-DL-aspartic acid (NMA), and kainic acid (KA)—directly into the adult rat diencephalon and found (82) that each produced an acute axon-sparing neurotoxic reaction with the severity of the acute reaction being directly proportional to the excitatory potency of the injected compound. Small doses (for example, 3.5 nmoles KA) were sufficient to destroy large numbers of neurons. We proposed, therefore, that these analogs might be used as lesioning agents for removing nerve cell bodies from a given brain region without damaging axons terminating in or passing through the region. We also noted that although many neurons were sensitive to the toxic effects of these compounds, some appeared to be either relatively or completely resistant (82). The latter phenomenon has long been recognized as a characteristic of Glu neurotoxicity (56,62,73). (For a series of recent studies addressing the selectivity and axon-sparing characteristics of KA neurotoxicity, see ref. 45).

Comparison of Glutamate and the More Potent Analogs

Recently, we compared the toxic activities of several excitotoxins on striatal neurons using the size of the tissue zone devoid of neurons 1 week following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intrastriatal injection as the index of neurotoxic potency (62,66). The order of potency (Fig. 5) for the excitotoxins as evaluated in this manner was KA > NMA > HCA > Glu, which is the known order of potency for the excitatory activities of these compounds. It is to be noted that the toxic activity of Glu is much weaker than that of KA. Indeed, the large doses of Glu required to induce substantial neuronal loss might lead one to suspect that the damage results from nonspecific factors, such as the hypertonicity of the injected solution. The fact that equally hypertonic solutions of GABA or NaCl induced no damage, however, argues against this conclusion (Fig. 5). The loss of striatal neurons following Glu injection and the failure of equimolar doses of GABA to destroy striatal neurons is illustrated in Fig. 6. Comparing the doses of KA (< 2 nmoles) and Glu (1,000 nmoles) required to induce roughly the same amount of striatal damage, it appears that KA is more than 500 times more powerful. This is very similar to the relative potencies of KA and Glu as neurotoxins when given systemically (46,77). Although the remarkably greater excitotoxic potency of KA compared to Glu is not fully understood, most investigators agree that protective mechanisms that inactivate Glu more efficiently than KA play an important role (see chapters by Johnston and McGeer, *this volume*). McGeer et al. (44) and Coyle et al. (16) have presented data suggesting a cooperation mechanism whereby the potency of KA is explained in terms of an ability of KA to act in concert with endogenous Glu to enhance the excitotoxic action of Glu at its natural synaptic receptor sites.

Excitotoxin-Induced Axon-Sparing Lesions

In the acute stages of the neurotoxic reaction induced by Glu or its excitatory analogs, dendritic and somal components of the neuron undergo extreme swelling and degenerative changes, whereas axons passing through or terminating in the region exhibit no changes. It was on the basis of such ultrastructural evidence that the Glu-type lesion was characterized as dendrosomatotoxic but axon-sparing (57,71,73,79,81). More recently, as the direct toxic action of potent Glu analogs, such as kainic acid, on various brain regions has been examined, additional evidence for the axon-sparing nature of the excitotoxic type of lesion has accumulated. In the kainate-lesioned striatum, for example, Coyle et al. (17) and McGeer and McGeer (43) have reported a marked loss of choline acetyltransferase and glutamic acid decarboxylase, enzyme markers for the intrinsic neurons of the striatum, without loss of tyrosine hydroxylase, an enzyme marker for extrinsic dopaminergic

FIG. 3. **a:** An axodendritic synaptic scene from the AH region of an infant mouse 30 min after a 3 mg/g s.c. dose of Glu. The presynaptic axonal component (A) appears normal, but the postsynaptic dendritic process (D) is massively dilated and contains scattered particles of debris, a multivesicle body, and a condensed, vacuolated mitochondrion undergoing degeneration. × 36,000. (From Olney, ref. 57.) **b:** A survey electron micrograph depicting the lateral edge of the AH from a 10-day-old mouse 6 hr after a 1 mg/g oral dose of Glu administered by feeding tube. Necrotic-neurons are present throughout the AH region. The typical pyknotic nuclei (*arrows*) and swollen cytoplasm (*double arrows*) are clearly evident. The smaller vacuous profiles (D) are massively dilated dendrites. × 900. (From J. Olney, *unpublished*.)

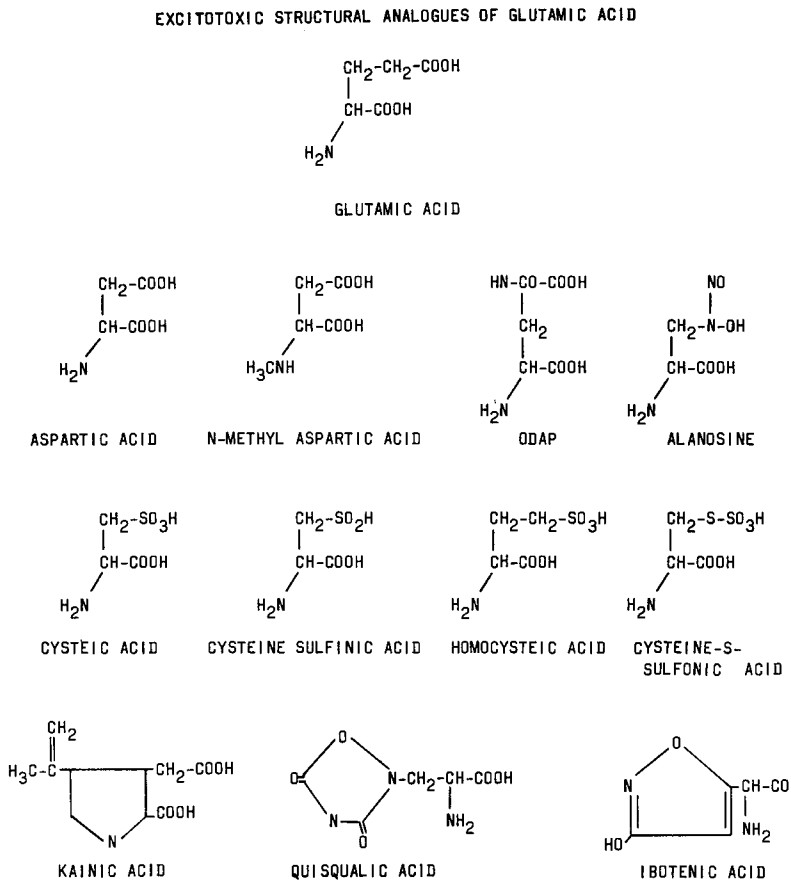


FIG. 4. Representative acidic amino acids known to have both neuroexcitatory and neurotoxic activity. ODAP (β-N-oxalyl-1-$\alpha\beta$-diaminopropionic acid) is an excitotoxin found naturally in chick peas; it is thought to be the neurotoxic factor responsible for the human crippling disease, neurolathyrism (74). Alanosine is an antibiotic and antileukemic agent recently demonstrated to be both a neurotoxin (65) and neuroexcitant (Curtis and Lodge, *personal communication*). Cysteine-S-sulfonic acid is an excitotoxin associated with the neurodegenerative metabolic disorder, sulfite, oxidase deficiency (73). For detailed information on other uncommon excitotoxins such as kainic, quisqualic, and ibotenic acids, please consult ref. 46. Thus far, we have not tested any excitatory analog of Glu and found it lacking in neurotoxic activity. The order of toxic potency as established in studies by us (58,60,65,69,71,73,74,77,80) or Coyle et al. (16) for these compounds is kainic > quisqualic = ibotenic > DL-N-methyl Asp > D-homocysteic = ODAP > alanosine = cysteine-S-sulfonic > L-homocysteic > cysteic = cysteine sulfinic = cysteic = L-Asp = D-Asp = D-Glu = L-Glu. Without significant exception, the same order has been described for the excitatory activities of these compounds (see chapters by Curtis and Johnston, *this volume*).

axons terminating in the striatum. In the hippocampus, Nadler et al. (48) have demonstrated histologically that KA deletes select intrinsic neuronal populations with subsequent degeneration of their axons while sparing axons of extrinsic origin terminating in the lesioned zone. We have examined several brain regions by

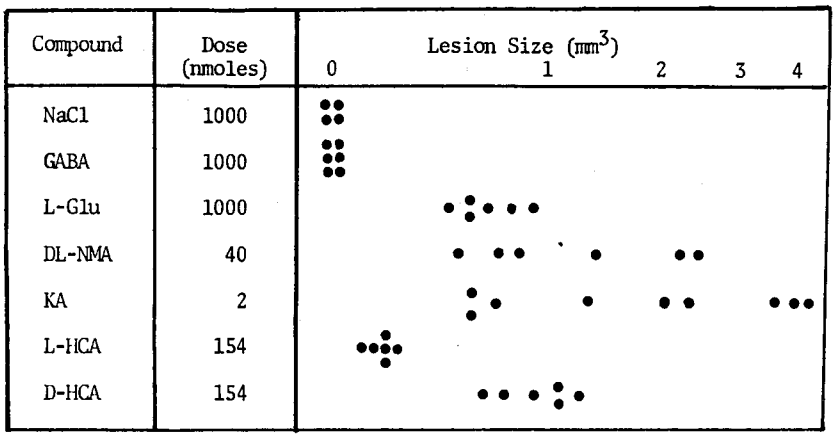


FIG. 5. NaCl, GABA, and several excitotoxins compared for striatal toxicity. All compounds were dissolved in sterile water and adjusted to neutral pH with NaOH. NaCl, GABA, and Glu were injected in 1-μl volume and the other compounds in 0.5-μl volume into the adult rat neostriatum. Animals were sacrificed by aldehyde perfusion fixation 1 week after injection and histological sections cut from araldite embedded blocks were evaluated for neuronal loss at the injection site. Since the shape of lesions tended to be eliptical, we established the margins of the lesion (area without neurons) at the site of injection and used a formula for the volume of an epipsoid ($v = \pi/6ab^2$ where a is the widest and b the narrowest diameter) to obtain a rough three-dimensional estimate of the total tissue mass devoid of neurons. Each dot represents a single animal. There was no detectable loss of neurons in NaCl or GABA injected brains. Since 2 nmoles KA destroyed more neurons than 40 nmoles NMA or 1,000 nmoles Glu, KA would appear to be > 20 and > 500 times more powerful than NMA and Glu, respectively. Comparison of the D- and L-isomers of HCA at the same dose revealed the D-isomer to be substantially more effective than the L-isomer in destroying striatal neurons.

electron microscopy 1 to 3 weeks after a KA lesion and have found numerous well-preserved axon terminals in zones that have sustained a total loss of intrinsic neurons (67,68). Such axons often retain postsynaptic densities that represent receptor membranes relinquished by the dying neurons to their prior presynaptic contacts (Fig. 7). Herndon and Coyle have observed the same phenomenon in the KA-lesioned cerebellar cortex (28).

Localization of the Toxic Mechanism to the Extracellular Compartment

In view of the well-known property of brain tissue to concentrate Glu from an incubation medium, the question arises whether cellular uptake of Glu with consequent derangement in intracellular metabolism could be the basis for Glu-induced neuronal necrosis. We attempted to study this recently (69) by taking advantage of the observation by Cox et al. (18) that D-HCA, a potent excitatory analog of Glu, is not taken up intracellularly by brain tissue, whereas L-HCA, a weaker excitant, is taken up by both high- and low-affinity transport systems. We compared the neurotoxicity of these stereoisomers by systemic administration to infant mice or direct injection into the striatum of adult rats, reasoning that if the D-isomer proved to be a more potent neurotoxin, this would imply a link between the toxic and

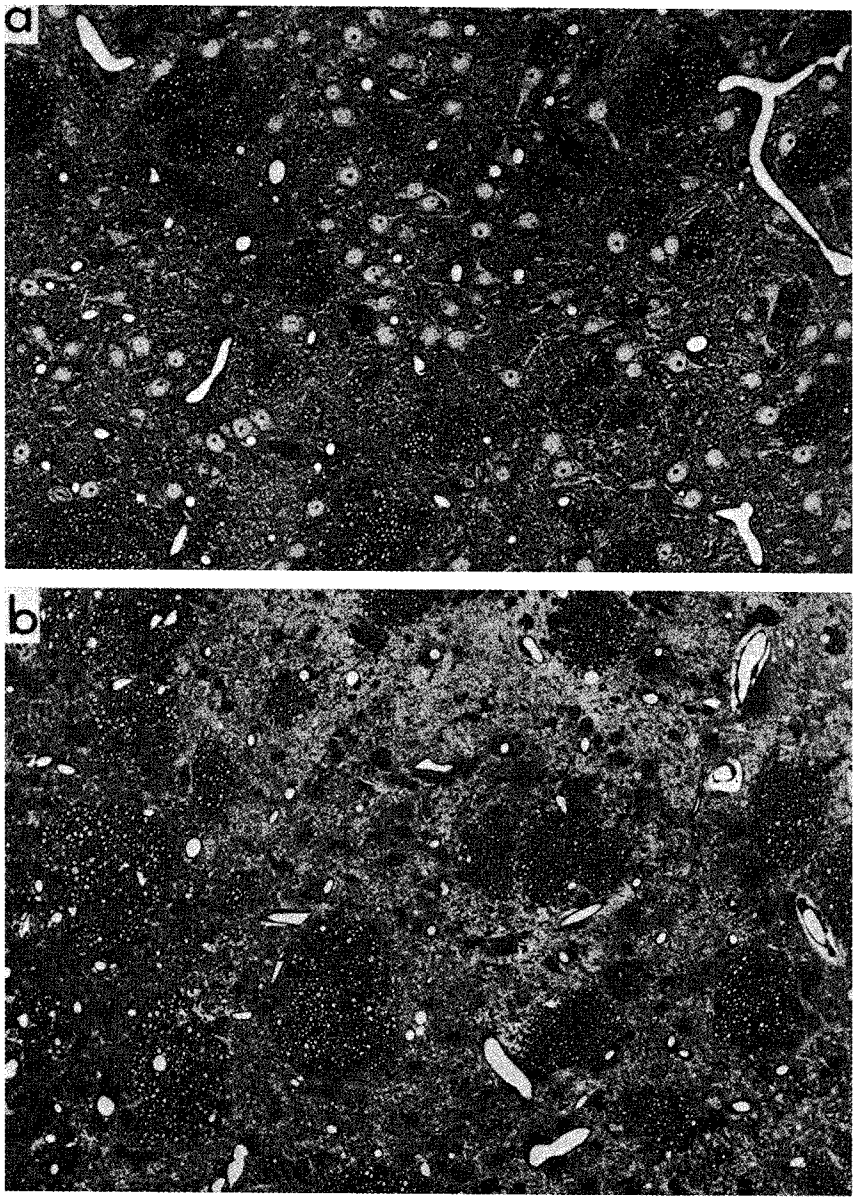


FIG. 6. **a:** The neostriatal scene appears entirely normal despite the injection of 1,000 nmoles GABA into this region 21 days previously. No neurons have been deleted. **b:** A comparable neostriatal scene near a site where 1,000 nmoles Glu were injected 21 days previously. All neurons have been eliminated from this striatal area, but axonal bundles appear quite normal. The numerous small dark bodies are glial cells, which typically proliferate at the scene of an excitotoxin-induced striatal lesion. × 266.

excitatory activities and identify both as actions exerted from the extracellular compartment. We found the D-isomer substantially more powerful than the L-isomer in necrosing AH neurons of the infant mouse (69) or striatal neurons of the adult rat (Fig. 5). This suggests that the toxic and excitatory activities of the HCA molecule are extracellularly mediated and argues against either phenomenon being dependent on intracellular uptake or the associated metabolic derangement. The same can be said for the KA molecule in view of recent evidence (Johnston, *this volume*) that this potent excitotoxin is not actively taken up into rat brain slices.

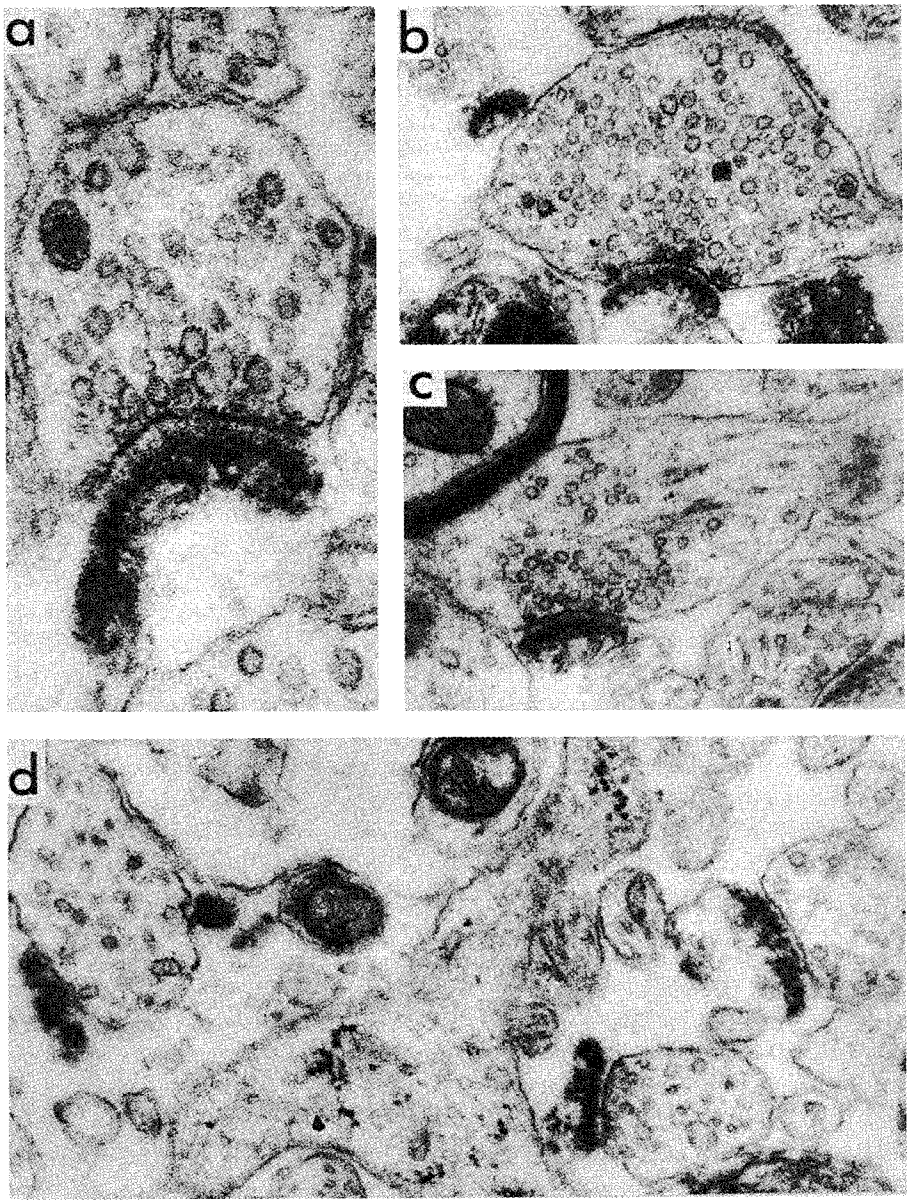
EXCITOTOXIC AMINO ACIDS AS NEUROENDOCRINE PROBES

The AH has long been recognized as an important, albeit poorly understood, neuroendocrine regulatory center. It is contiguous with the ME and the infundibular stalk that connect the pituitary gland to the brain. AH neurons have short axons that are thought to terminate in the external mantle of the median eminence (EMME) on or near portal vessels that convey various regulatory factors (hypophysiotrophic hormones) from the EMME zone to the adenohypophysis. Other hypothalamic and possibly extrahypothalamic centers also contribute axons to the EMME region, and this had made it difficult, in the absence of specific tools, to delineate the endocrine regulatory roles of individual centers such as AH. Fortunately, excitotoxic amino acids have properties that make them very promising tools for probing the neuroendocrine functions of AH neurons.

For an agent to serve as a useful systemic neuroendocrine probe, it must have access from blood to the endocrine hypothalamus. Perez and Olney (85), applying quantitative histochemical methods to obtain a microregional delineation of Glu uptake patterns, demonstrated a fourfold elevation of Glu in the AH-ME following a 2 g/kg (s.c.) dose of Glu administered to immature mice, but detected no changes in Glu concentrations in other hypothalamic or thalamic regions samples. Moreover, the time course of Glu accumulation in the AH-ME paralleled the time course of lesion formation in that region (55,57). Since it is well established that the systemic administration of high doses of Glu results in little or no change in whole brain Glu concentrations (36), it is quite clear that the homeostatic mechanisms regulating net Glu influx from blood into the AH-ME are very different from those pertaining to brain proper. Furthermore, since various acidic excitatory analogs of Glu mimic the selectivity of Glu in damaging the AH when administered subcutaneously (65,71,73,77), these compounds presumably share with Glu the ready access it has to AH-ME neurons. Selective (net) uptake into the AH coupled with the dual capacity to either stimulate the firing of AH neurons or destroy them makes these agents ideal for use as either provocative or ablative neuroendocrine investigational tools.

ABLATION APPROACH

The first report (55) of Glu-induced brain damage was accompanied by a brief description of the neuroendocrine disturbances that animals treated in infancy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typically manifest as adults, and it was postulated that all or nearly all such disturbances stem from the loss of AH neurons (Fig. 2). This interpretation, if correct, suggests that a full characterization of the endocrine deficiency syndrome associated with Glu treatment should provide valuable clues to the endocrine regulatory functions subserved by AH neurons. The major features of the syndrome as originally described (55) were obesity, skeletal stunting, adenohypophyseal hypoplasia, female sterility, and pathomorphological changes in the reproductive organs of the female. Available evidence confirming and expanding on each feature will be summarized.

Pituitary Status

Olney noted (55) that although Glu-induced damage to the infant hypothalamus was not accompanied by acute pathological changes in the pituitary gland, the anterior lobe of the pituitary was characteristically very small when treated animals attained adulthood (Fig. 8). The severe hypoplasia of the anterior pituitary was ascribed to the removal of trophic influences ordinarily exerted on that organ by AH neurons. A markedly undersized anterior pituitary in both male and female adult animals (mice, rats, and hamsters) following neonatal Glu treatment has now been described by numerous researchers (14,30,37,49,51,53,55,87,92,96,103). The pituitary content of growth hormone (GH), luteinizing hormone (LH) and prolactin (Prl) in Glu-treated rodents is reduced either commensurate with or in excess of the reduction in mass of the anterior pituitary (Table 1) (49,92). When adult rats treated neonatally with Glu are challenged with appropriate releasing agents, however, normal or supranormal LH, TSH, or Prl responses are elicited (14,53), indicating unimpaired ability of the pituitary to release trophic hormones.

Obesity

In mice treated in infancy with either single or multiple subcutaneous injections of Glu, Olney (55) described an obesity syndrome in which treated animals, initially

FIG. 7. **a–c:** The synaptic complexes depicted are characteristic of those abundantly present in the striatum 1 to 3 weeks after a 10-nmole KA injection. They appear to be asymmetric synapses of the type that axon terminals ordinarily make with dendritic spines and shafts of striatal neurons. The presynaptic element appears healthy and contains numerous synaptic vesicles. The synaptic cleft remains distinct and has postsynaptic dense material extending from it that does not appear fundamentally different from the postsynaptic web of an intact synapse. **a,** × 96,000; **b,c,** × 45,000. (From Olney and de Gubareff, ref. 67.) **d:** The synaptic complexes characteristic of those seen in the olfactory cortex 1 week after KA administration. It was recently demonstrated (68) that olfactory cortical neurons are so sensitive to KA toxicity that they selectively degenerate following small doses of KA injected by any of several routes of administration (intradiencephalic, intrastriatal, intraventricular, or subcutaneous). The olfactory cortex is innervated by axons from the olfactory bulb, which putatively use Glu as transmitter. The terminals of these neurons are depicted here with postsynaptic receptor membranes remaining attached to them after KA-induced degeneration of the olfactory cortical neurons that previously housed these membranes. × 36,000. (From Olney and de Gubareff, ref. 68.)

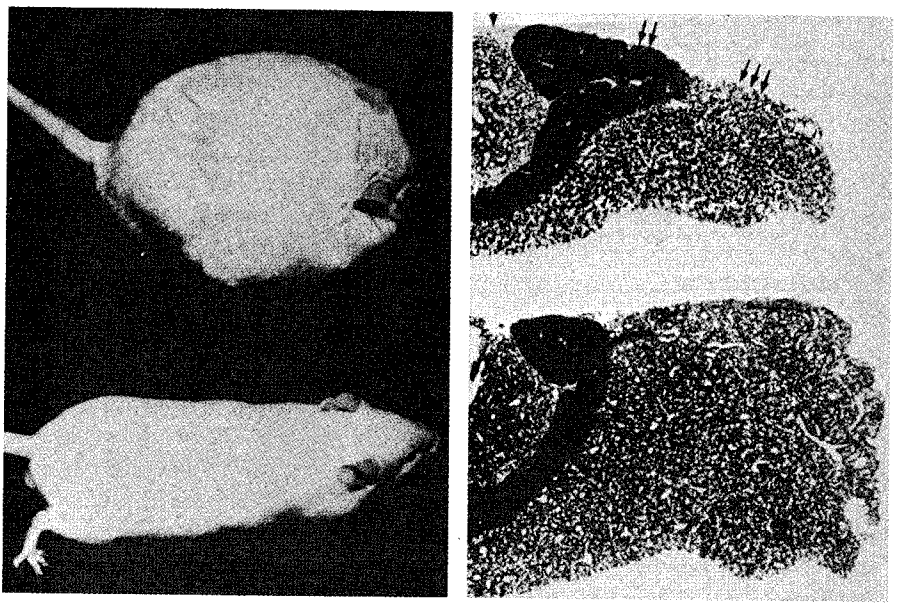

FIG. 8. **Left:** Two 9-month-old male litter mates. The experimental mouse (*left*) received daily subcutaneous Glu injections on postnatal days 1 to 10 and at 9 months weighed 84 g. The untreated control animal (*right*) weighed 44 g. In addition to obesity, note that the experimental animal is short and its body coat is not as sleek as that of the control. (From Olney, ref. 55.) **Right:** Directly across from each animal is its pituitary gland. Only ½ of each pituitary is shown. The anterior lobe (*triple arrows*) of the Glu-treated animal is much smaller than normal. The intermediate lobes (*double arrows*) of the two pituitaries are the same size, although one looks larger because it was sectioned in a slightly different plane. The posterior lobes (*single arrow*) are the same size. Pituitaries, × 38. (From Olney and Price, ref. 75.)

lower in body weight, surpassed the weight of litter mate controls at about 45 days of age and thereafter continued to amass considerable carcass fat at a slow but steady pace throughout adulthood (Fig. 8). Measurement of food intake revealed the treated animals to be slightly hypophagic compared to controls and they appeared lethargic (55). Many researchers have now confirmed Glu-induced obesity in either mice (2,9,12,20,86,89) or rats (35,51,54,92). Bunyan et al. (9), finding Glu treatment of newborn mice to be almost 100% reliable in inducing a high degree of obesity, stressed the potential value of the Glu-obese mouse as a model for studying obesity. Cameron and colleagues (12,89) administered Glu to KK mice, an inbred strain with a high genetic susceptibility to diabetes, and found (89) that it unmasked diabetes; treated animals not only became markedly obese, but developed hyperglycemia accompanied by gross hyperinsulinemia, implying a state of insulin resistance. They considered the hyperglycemia and hyperinsulinemia to be a direct result of the hypothalamic abnormality induced by Glu in this diabetes-prone strain and suggested that further study of this model may shed light on the role of the hypothalamus in obesity and diabetes.

In Glu-treated rats, Knittle and Ginsberg-Fellner (35) established significant

TABLE 1. *Endocrine organ weights and hormone values in Glu-treated rodents*

Animal	Exp.	*N*	Pituitary (mg)	Testes/ Ovaries (mg)	Adrenals (mg)	Pituitary GH (μg)	Plasma GH (ng/ml)	Plasma corticosterone (μg/100 ml)
Mice								
♂	Glu	9–12	0.54 ± 0.08^a	167.5 ± 16.7^a	8.1 ± 0.5^b	8.7 ± 1.2^a	5.3 ± 0.6^a	37.0 ± 4.0^a
	Control	10–16	2.04 ± 0.08	282.0 ± 7.4	9.0 ± 0.5	249.3 ± 14.9	122.7 ± 32.0	5.7 ± 1.3
♀	Glu	8–11	0.86 ± 0.07^a	12.9 ± 2.3^a	11.4 ± 1.1^b	15.1 ± 2.6^a	3.7 ± 0.9^a	35.2 ± 5.3^c
	Control	9	2.94 ± 0.14	39.1 ± 8.1	8.8 ± 0.4	269.2 ± 18.9	17.7 ± 2.5	12.4 ± 1.9
Rats								
♂	Glu	7–26	5.62 ± 0.23^a	2.67 ± 0.10^a	38.2 ± 4.0^b	527.0 ± 77.0^a	21.0 ± 4.5^a	6.2 ± 1.2^b
	Control	7–26	8.75 ± 0.43	3.35 ± 0.13	43.2 ± 3.3	909.0 ± 91.0	110.5 ± 18.9	6.2 ± 1.6
♀	Glu	6–18	7.74 ± 0.40^a	93.9 ± 5.5^a	38.0 ± 4.0^d	327.0 ± 16.0^a	13.2 ± 1.9^a	31.0 ± 5.4^c
	Control	8–19	12.28 ± 0.49	127.3 ± 4.8	59.0 ± 5.1	618.0 ± 56.0	34.9 ± 4.4	10.3 ± 2.7

Glu was given to all experimental animals s.c. at graduated doses from 2.2 to 4 mg/g in 5 daily treatments from days 2 through 7 postnatally. Animals were sacrificed at 5 months of age.

[a] $p < 0.001$; [b] Not significant; [c] $p < 0.01$; [d] $p < 0.02$.

From B. Massey, K. Kipnis, and J. Olney, *unpublished*.

obesity by measuring the weight of epididymal fat pads 5 months after treatment of rat pups. The increase in the weight of fat pads was traced to an increased lipid content per cell rather than an increased number of adipose cells. Also noted was a decreased responsiveness to the lipolytic effect of epinephrine and an increased responsiveness to the antilipolytic effects of insulin.

All researchers who have attempted to measure food intake in Glu-treated obese animals have reported them to be either hypophagic or normophagic, but never hyperphagic (2,9,20,51,54,55,92). Activity levels of Glu-obese rodents have been reported as increased (2), unchanged (54), and decreased (51,86) compared with controls, so that it remains unclear what role energy expenditure plays in Glu obesity.

Effects on Somatotrophin Axis

An approximate 10% reduction in linear bone growth in Glu-treated mice (Fig. 8), initially established by skeletal X-ray measurements (55), has been confirmed in both mice (2,9,30,31,86,89,50) and rats (51,54,92,102). Marked reductions in pituitary GH content and basal serum levels of GH (Table 1) have also been a consistent finding in both mice or rats treated neonatally with Glu (14,53,92,102). Terry et al. (102), by studying serum GH patterns over time in individual rats treated neonatally with Glu, have established that the pulsatile output of GH is markedly diminished. Reduced tissue levels of somatostatin (SRIF) in two specific brain regions, the mediobasal hypothalamus (14,53,102) and basolateral amygdala (102)—regions known to be involved in regulating GH secretion—have also been reported in Glu-treated rats.

Effects on Gonadotrophin Axis

Initial observations on Glu-treated mice (55) suggested disturbances in the neural-gonadal axis of only the female, but subsequent studies have extended the finding to include both female and male mice, rats, and hamsters (14,30,37,49, 51,87,92,103). Holzwarth-McBride et al. (30) and Pizzi et al. (87) treated large numbers of mice neonatally with Glu. In both studies, treated animals had significantly reduced weights of testes and ovaries, and reproductive capacity was significantly impaired in both sexes. Delayed vaginal opening and disturbed estrous cycles have also been reported in Glu-treated female mice (49,87).

In Glu-treated rats, the ovaries (14,51,92,103), uteri (51), testes (14,51,92,103), and seminal vesicles (14) were reportedly smaller than controls, although testicular weight differences were significant in some studies only when given as absolute weights, uncorrected for body weight. Estrous cycle disturbances have been observed in Glu-treated rats (14,103), but abnormal reproductive capacity has not been demonstrated. A marked decrease in the pituitary content of LH (92) and a decrease in basal serum LH (14,51) were found in both male and female Glu-treated rats, although the depressed serum LH levels were not considered statistically significant in one study (51).

Lamperti and Blaha (37) administered Glu subcutaneously to neonatal hamsters in doses of either 4 or 8 mg/g and observed a reduction in the weights of testes, seminal vesicles, ovaries, uteri, and anterior pituitaries in all treated animals. In hamsters given 8 mg/g, all females were acyclic, with ovaries containing only secondary-stage follicles, and males had markedly atrophic testes that were devoid of spermatids and spermatozoa in the seminiferous tubules. Whereas such extreme changes following 8 mg/g treatments might relate to the destruction of AH neurons, it must not be forgotten that exceedingly high doses of Glu, in the mouse and rat at least, destroy preoptic hypothalamic neurons that are believed to regulate the ovarian cycling in the female rodent (24).

Effects on Prl

Nagasawa et al. (49) administered a single dose of Glu (4 mg/g) to neonatal female mice and observed that treated animals in adulthood had a reduced pituitary content of Prl and suppressed development of mammary systems. In rats treated neonatally with Glu, Clemens et al. (14) elicited serum Prl peaks in response to 5-hydroxytryptophan challenge that were significantly higher than controls. In ovariectomized rats treated neonatally with Glu, estradiol benzoate treatments induced large Prl elevations in controls, but very slight Prl responses in Glu-treated animals.

Thyroid Status

The apparent lethargy and roughness of body coat noted by Olney (55) in Glu-obese mice suggested a thyroid-deficient state. Nemeroff et al. (53), who are the only researchers to report thyroid function data on Glu-treated animals, considered their Glu-treated rats hypothyroid on the basis of a significantly lower serum triiodothyronine and free thyroxine index compared to controls.

Adrenal Status

Several groups have reported that the adrenal weights of MSG-treated animals were smaller than in controls, but usually not significantly so. The adrenal weights given in Table 1 are fairly characteristic. Of greater potential interest, however, is the recent observation of B. Massey, D. Kipnis, and J. Olney (*unpublished*) that Glu-treated mice and female rats have exceedingly elevated basal corticosterone levels (Table 1).

Mediobasal Hypothalamus: Putative Transmitters and Hypophysiotropic Factors

Nemeroff et al. (51) have reported the loss of dopamine (DA) histofluorescence in AH cell bodies of rats treated neonatally with Glu, but no appreciable change in the intensity of DA histofluorescence in the EMME, where DA neurons of AH are

thought to terminate. Helme et al. (27) described the loss of DA histofluorescence in the EMME of Glu-treated mice, but not rats. Holzwarth-McBride et al. (30) noted reduced DA histofluorescence in both AH and EMME of Glu-treated mice. Employing the Palkovits microdissection approach, Nemeroff et al. (53) found that the Glu-lesioned rat AH has normal tissue concentrations of luteinizing hormone-releasing hormone (LHRH), thyrotropin-releasing hormone (TRH), serotonin, and norepinephrine, but greatly reduced concentrations of DA and choline acetyltransferase, an enzyme marker for cholinergic neurons. Clemens et al. (14) recently confirmed a 60% reduction in the DA concentration of the mediobasal hypothalamus in Glu-treated rats. Lechan et al. (38) reported no reduction in LHRH concentrations and no loss of immunohistochemical staining for LHRH in Glu-lesioned mouse hypothalamus.

Holzwarth-McBride et al. (30) and Paull and Lechan (84) examined the EMME ultrastructurally after destroying an estimated 80% of the AH neurons by Glu treatment of immature mice. Neither group was able to find significant signs of axon terminal degeneration or change in the makeup, size, or configuration of the ME. Olney et al. (78) conducted a similar study in which the AH neurons were destroyed by Glu treatment of either infant or adult mice, and the EMME was examined at numerous posttreatment intervals as AH neurons were acutely degenerating and being phagocytized. At approximately 16 hr posttreatment, they detected axon terminal degeneration in the EMME region, but the numbers of degenerating processes were so few that they concluded that AH neurons are only a minor source of the fibers that project on the EMME (78).

Other Excitotoxins Used As Ablative Neuroendocrine Tools

Schainker and Olney (50) explored the potential of other excitotoxins (L-aspartate and L-cysteate) to reproduce the endocrine disturbances associated with Glu treatment in mice. Animals treated with cysteate and aspartate (Asp) developed obesity, skeletal stunting, and decreased weights of the adenohypophyses, ovaries, and testes, whereas NaCl-treated animals were free from such stigmata. Pizzi et al. (88) confirmed the above in Asp-treated mice and, in addition, established that Asp treatment results in impaired reproductive capacity of both sexes.

PROVOCATIVE APPROACH

If excitatory amino acids in toxic systemic doses destroy AH neurons by an excitatory mechanism, as proposed above, it follows that the same compounds in subtoxic doses—that is, doses that do not damage AH neurons—may excite them to fire at an accelerated rate, just as the firing of other central neurons is stimulated by the microelectrophoretic application of these compounds. Since bioelectric discharge is presumably the first step in the chain of events through which AH neurons influence pituitary hormonal outputs, increased AH discharge activity should give rise to altered hormonal outputs. Experiments recently undertaken to explore this proposition will be summarized.

Acute Effects of Glu on LH, GH, and Prl

In 1976 we reported (63,64) that the subcutaneous administration of Glu to adult male Holtzman rats at 1 mg/g, approximately 25% of the dose required to destroy AH neurons in the adult rat, results in significant elevations of serum LH and testosterone 15 min after Glu treatment. Subsequently, we observed that the peak LH response to Glu occurs even more rapidly—in the 5- to 10-min posttreatment interval (Fig. 9). Therefore, we have focused upon the 7½-min interval in our more recent stimulated LH output studies (see below). Terry et al. (102) administered Glu, 1 mg/g s.c., to adult male rats and observed an acute elevation of serum Prl 15 min after treatment and a sustained (for 5 hr) suppression of the pulsatile output of GH.

Acute Effects of Potent Glu Analogs on Serum LH

If the hypothesis is correct that Glu in subtoxic doses induces acute elevations in LH by stimulating bioelectric activity of AH neurons, it should be possible to elicit a similar LH response with subtoxic doses of other neuroexcitatory analogs of Glu. To test this proposal, we administered HCA, NMA, and KA, three potent excitatory analogs of Glu, to 25-day-old male rats in doses ranging from 1 to 100 mg/kg and determined serum LH levels 7½ min after subcutaneous injection of each excitant (91). Each excitant elicited a rapid and striking LH elevation, the lowest effective doses being 5, 16, and 50 mg/kg for KA, NMA, and HCA, respectively (Fig. 10).

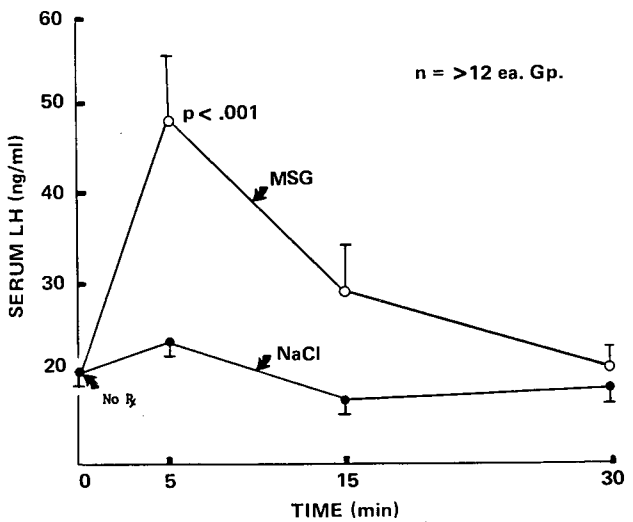

FIG. 9. Serum LH levels (mean ± SEM) of adult male rats that received 1 g/kg s.c. Glu 5 to 30 min before sacrifice. A rapid rise in serum LH levels occurred within 5 min of Glu administration. By 30 min, LH values were the same for experimental and untreated or NaCl-treated control animals. The latter received 0.35 g NaCl/kg, the molar dose equivalent of 1 g/kg Glu. (From Olney and Price, ref. 75.)

Thus, the order of potency observed for the LH-stimulating activities of these agents (KA > NMA > HCA) was the same as had previously been shown for both their excitatory and toxic activities. We also evaluated the brain-damaging potential of these compounds on 25-day-old male rats and found that the lowest doses of NMA and HCA effective in producing AH-ME damage were 3 to 4 times higher than the lowest doses required to induce LH elevations (91). For KA, however, there was little or no practical margin between a brain-damaging and LH-stimulating dose; moreover, we found that KA tended to damage several extrahypothalamic regions of brain more readily than it damaged AH. Thus, we judged NMA and HCA highly promising but KA relatively unsuitable for neuroendocrine investigational purposes.

GABA or Taurine Blockade of NMA-Stimulated LH Release

In the early work of Curtis and Watkins (19), it was observed that when applied iontophoretically, the neuroinhibitory amino acids such as β-alanine, GABA, and taurine were effective in blocking the excitatory responses of central neurons to excitants such as Glu, Asp, or cysteate. To test the hypothesis that excitatory amino acids induce LH release by excitation of AH neurons, we administered NMA to 25-day-old rats in sufficient dosage (25 mg/kg) to assure a reliable LH response and attempted to block this response by simultaneous administration of GABA or

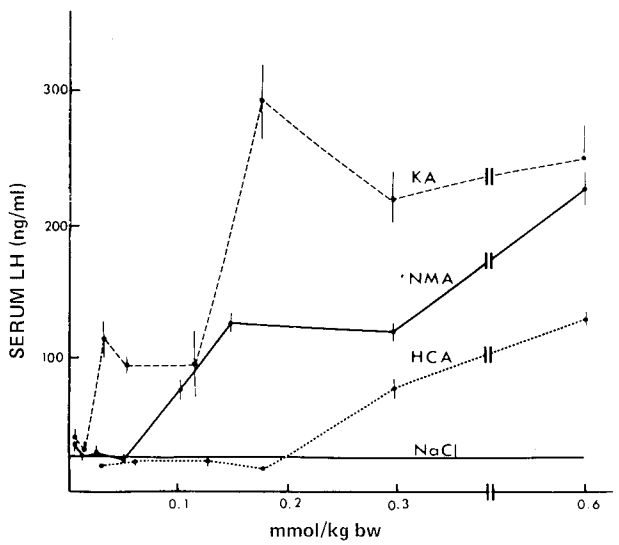

FIG. 10. A total of 255 male rats, 25 days old, were treated with various doses of HCA, NMA, or KA, and serum LH levels were determined 7½ min later. KA induced significant ($p < 0.001$) elevations beginning at a dose of 5 mg (0.03 nmoles)/kg, NMA at 16 mg (0.1 nmoles)/kg, and HCA at 50 mg (0.3 nmoles)/kg. The horizontal line above the *abscissa* represents pooled base-line values determined on untreated or NaCl-treated control rats. (Modified from Price et al., ref. 91.)

taurine. We also studied the effects of DA blocking agents (pimozide and chlorpromazine) in the same study. The results (Fig. 11) of these experiments were unequivocal. Neither GABA nor taurine by itself influenced serum LH concentrations in the interval tested (7½ min following injection), but when given with NMA, either compound completely blocked the striking 5- to 10-fold LH elevations typically observed in rats 7½ min after NMA treatment. Pimozide and chlorpromazine were ineffective in blocking the NMA-induced release of LH.

Blockade of NMA Activity by α-Aminoadipate

In very recent experiments we have administered α-amino-DL-adipate (α-AA) together with NMA in an effort to block either the LH-releasing activity or the

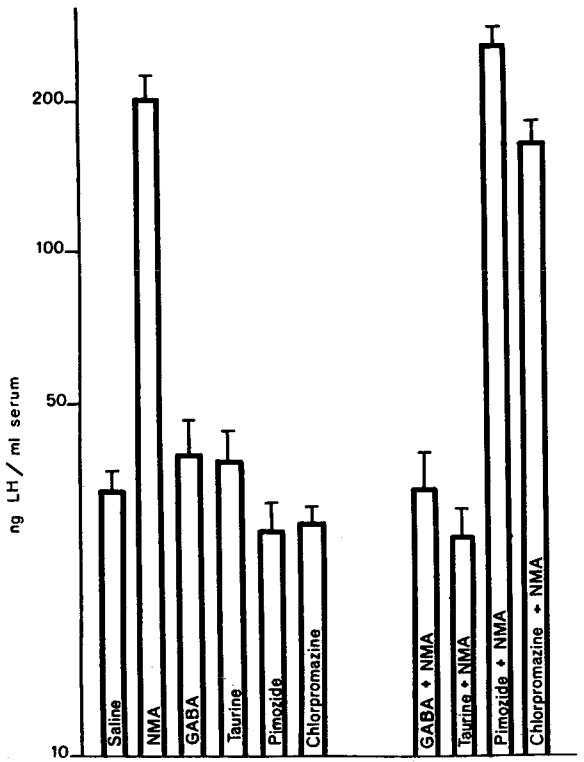

FIG. 11. Serum LH levels (mean ± SEM) 7½ min after subcutaneous treatment of 25-day-old male rats with various agents in the following doses: 25 mg/kg NMA, 1 g/kg GABA, 1 g/kg taurine, 0.6 mg/kg pimozide, 25 mg/kg chlorpromazine. When given alone, NMA induced a significant LH elevation ($p < 0.001$), which was effectively blocked by GABA or taurine, but not by pimozide or chlorpromazine. When GABA, taurine, pimozide, or chlorpromazine were administered without NMA, LH values were not significantly different from saline control values. In drug combination experiments, GABA or taurine were administered concurrently with NMA, but pimozide and chlorpromazine were given as multiple pretreatments 24, 14, and 2 hr prior to NMA. (From Olney and Price, ref. 75.)

neurotoxic effects of NMA. Our preliminary findings are that α-AA effectively antagonizes the LH-releasing action of NMA in 25-day-old rats (M. T. Price and J. W. Olney, *unpublished*) or the toxic action of NMA on AH neurons of the adult mouse (J. W. Olney, *unpublished*). The impetus for conducting such studies came from the recent microelectrophoretic experiments of Biscoe et al. (7) showing that α-AA specifically antagonizes the depolarizing action of NMA on central mammalian neurons. Although our findings with α-AA are very preliminary, we believe them worth mentioning because of the distinct possibility that α-AA, unlike GABA or taurine, specifically antagonizes the action of NMA at its excitatory receptor locus. Although GABA and taurine are very effective in blocking the LH-releasing action of NMA, they are totally ineffective in antagonizing the neurotoxic activity of NMA (J. W. Olney and T. Fuller, *unpublished*). We interpret this as evidence that GABA and taurine act at a different receptor site than NMA—a site that aborts the action potential of AH neurons and hence the LH-releasing action of NMA, without preventing its action at excitatory receptors on the dendritic and somal surfaces of AH neurons where the initial excitatory stimulus is translated into a toxic effect. Blockade of both the LH-releasing action and AH neurotoxic effects of NMA by α-AA, in light of evidence that α-AA is a specific antagonist of NMA-induced excitation, strongly suggests that NMA-induced excitation of AH neurons is the triggering mechanism underlying both the LH releasing and neurotoxic actions of NMA.

NEUROENDOCRINE DATA SUMMARIZED

For ablation purposes, it is possible to delete about 80 to 90% of the neurons of AH by treating rodents in infancy with subcutaneous Glu or certain excitatory analogs of Glu. Rodents thus treated, including mice, rats, and hamsters, manifest a complex syndrome of endocrine-type disturbances, including normophagic obesity, skeletal stunting, impaired reproductive capacity (not established in rats), reduced mass of the anterior pituitary and gonads, and reduced pituitary content of GH, Prl, and LH. Pulse amplitude of serum GH and basal serum levels of GH and probably LH are also depressed. Responsiveness of hypothalamic Prl release mechanisms to estrogenic and serotonergic stimuli are altered. In Glu-treated rats, serum triiodothyronine and free thyroxine indices are in the hypothyroid range and serum corticosterone levels are markedly elevated in Glu-treated male and female mice and female rats. The mechanism by which Glu treatment results in obesity remains a mystery, although the AH lesion is probably responsible since treatment with other Glu analogs that reproduce the AH lesion also cause obesity.

Analysis of the mediobasal hypothalamus following Glu treatment suggests that in terms of neurotransmitter content, the deleted AH neurons are composed of at least two subpopulations—dopaminergic and cholinergic. Whether any AH neurons contain LHRH remains unclear, but Glu ablation studies suggest that if there are LHRH-containing AH neurons, they do not supply more than a small percentage of the total LHRH-containing fibers that terminate in the EMME region. Although

evidence also remains incomplete regarding the contribution by AH of DA-containing fibers to the EMME region, it appears likely that AH contributes only a portion of such fibers and that the remainder come from extra-AH sources that have yet to be identified.

Reduced tissue levels of SRIF in the mediobasal hypothalamus and basolateral amygdala have been reported in Glu-treated rats. Since Glu does not directly damage the amygdala, loss of SRIF in amygdala may reflect atrophy of the SRIF system through disuse, i.e., the AH lesion may break circuits that promote GH secretion, leaving the animal in a GH-deficient state in which SRIF inhibitory circuits are unneeded and unused. Alternatively, AH neurons may be the target cells upon which SRIF-containing fibers from the amygdala project. Removal of the target cells may cause retrograde degeneration of the SRIF system (or failure of the system to fully develop).

Evidence pertaining to the acute effects of systemically administered excitotoxins on neuroendocrine systems (provocative approach), although only preliminary, suggests that subtoxic doses of Glu stimulate a burst of LH and Prl output, but suppress GH pulsatile output in adult male rats. The most potent excitatory analogs of Glu (HCA, NMA, and KA) are effective in stimulating LH output in direct proportion to their excitatory potencies. NMA, being particularly reliable and potent in stimulating LH output and lacking the erratic toxicity of KA, may be an ideal probe for studying LH release mechanisms. It has been demonstrated that NMA-induced LH release is totally blocked by simultaneous subcutaneous administration of GABA or taurine, but is unaffected by pimozide or chlorpromazine (dopamine receptor blocking agents). Failure of the latter agents to influence NMA-induced LH release tends to exclude dopaminergic neurons as the subpopulation of AH neurons through which NMA induces LH release. Future research should include studies designed to determine whether this phenomenon is mediated by cholinergic AH neurons.

The demonstration by Clemens et al. (14) that rats with AH lesions have a weak Prl response to estrogenic stimulation suggests that AH neurons may be a major link in an estrogen feedback loop for Prl regulation. Whether this feedback loop involves the DA subpopulation of AH neurons remains to be clarified, as does the exaggerated Prl response of the Glu-lesioned rat to serotonergic stimuli (14) and the acute burst of Prl output reported following Glu administration to normal male rats (102).

PROSPECTS FOR PRIMATE NEUROENDOCRINE STUDIES

Others have reported failure to demonstrate brain damage from Glu administration in primates (see chapters by Worden and Reynolds, *this volume*); such evidence is subject to the interpretation that primates are not susceptible to the mechanism of Glu neurotoxicity. If this were so, it would limit the usefulness of excitatory amino acids as neuroendocrine probes. We believe primates are quite susceptible, however, since we were able to locate Glu-type lesions in the AH in all six infant rhesus monkeys treated either orally or subcutaneously with Glu, but found no cytopathol-

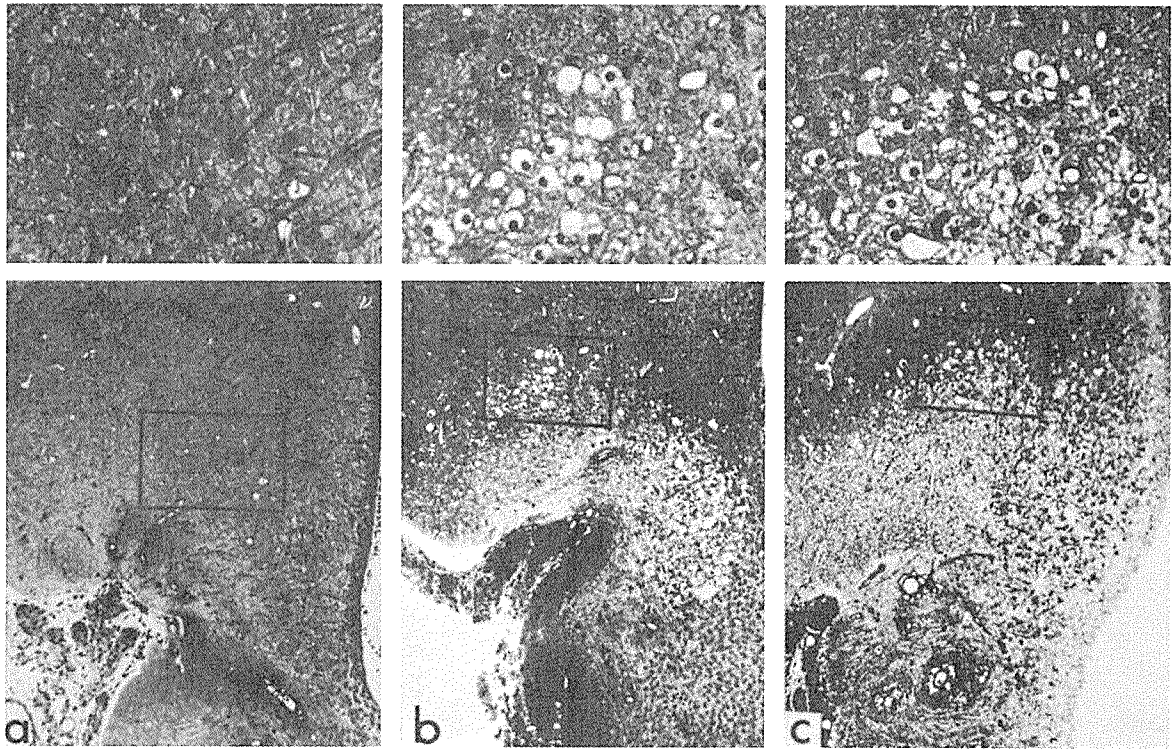

FIG. 12. The AH and infundibular region of 3 rhesus monkey hypothalami are depicted in the lower figures with magnified views (*boxed areas*) of AH presented above. **a:** From a control infant rhesus monkey given NaCl s.c. at 24 mmoles/kg (the molar equivalent of 4 g/kg Glu). The tiny dark profiles lining the ventricle are red blood cells, which reflect the hemorrhagic consequences of administering such a high solute load to an infant monkey. The hypothalamus is histologically normal. **b:** From an experimental infant monkey given Glu s.c. at 2.7 g/kg. The acute edema sweeping across the AH region and numerous acutely necrotic neurons (bull's eye profiles) in the middle of AH—the characteristic signs of a Glu-type lesion in any species—are clearly evident. **c:** From a fetal rhesus monkey following administration of a 2 g/kg *i.v.* dose of Glu to the pregnant mother (152nd day of gestation). The fetus was removed by cesarean section and sacrificed 5 hr following maternal Glu administration. The typical signs of a Glu lesion are unmistakably present in the AH region of this fetal hypothalamus. Lower panel, × 72; upper panel, × 216.

ogy in any of three rhesus monkey controls treated with NaCl (Figs. 12 and 13) (79,81). Furthermore, we recently completed a more extensive evaluation of these monkey brains and found typical Glu-type lesions in the AP of Glu-treated animals, but no such cytopathology in controls (Fig. 14). In addition, we administered (59) Glu intravenously to a pregnant rhesus monkey in late gestation (152 days), delivered a viable fetus by cesarian section 5 hr later, examined its brain by light and electron microscopy, and found sizeable acute Glu-type lesions in both the AH-ME and AP (Figs. 12 and 14); the mother also sustained similar damage from the high intravenous load of Glu (Fig. 14).

In light of the above, we believe excitatory amino acids may prove to be valuable neuroendocrine investigational tools for use with subhuman primates. Ablational studies could be undertaken with Glu treatments being given either pre- or postnatally. Since monkeys are scarce and costly, however, we believe that the emphasis should be on the provocative rather than ablative approach, the advantage of the former being that numerous experiments can be performed on a single animal. The observation that Glu destroys AP neurons in monkeys is of considerable interest in that Glu is known to have emetic properties in several species (42,81,104), including humans (40), and AP neurons are believed to subserve an emesis chemoreceptor trigger function (8). Glu may prove useful as either a provocative or ablative tool for studying the role of AP neurons and of neurotransmitter mechanisms in emesis chemoregulation in subhuman primates.

EXCITOTOXINS AS FOOD ADDITIVES: SAFETY IMPLICATIONS

Glu has long been used as a food-flavoring agent and currently remains among the additives listed by the Food and Drug Administration (FDA) as GRAS (generally regarded as safe). In the absence of regulatory restrictions, Glu was added liberally to processed infant foods for many years. In 1969, when the potential of Glu to induce brain damage in infant animals following oral administration was demonstrated, baby food manufacturers voluntarily stopped adding Glu to baby foods. About the same time, however, they began adding protein hydrolysates (rich in Glu and aspartate) in concentrations sufficient to maintain the free Glu content in baby foods at flavor levels to which the maternal palate had been conditioned. In addition, babies continued to be fed Glu-supplemented processed foods from the adult table. In 1973, at FDA request, the Federated American Societies for Experimental Biology (FASEB) formed an 11-member scientific advisory committee to review the safety of GRAS food additives. In 1976 this Committee advised FDA that neither Glu nor protein hydrolysates could be considered safe for use in baby or junior foods (98). The FASEB Committee is now finalizing a set of recommendations that presumably will guide the FDA in making a regulatory decision. Concerning the much-contested issue of primate susceptibility to Glu-induced brain damage, this Committee evaluated all relevant evidence and expressed the following opinion, "Despite the fact that the brain damage reported in neonatal mice, rats and monkeys by some research groups could not be confirmed by other groups, the Select

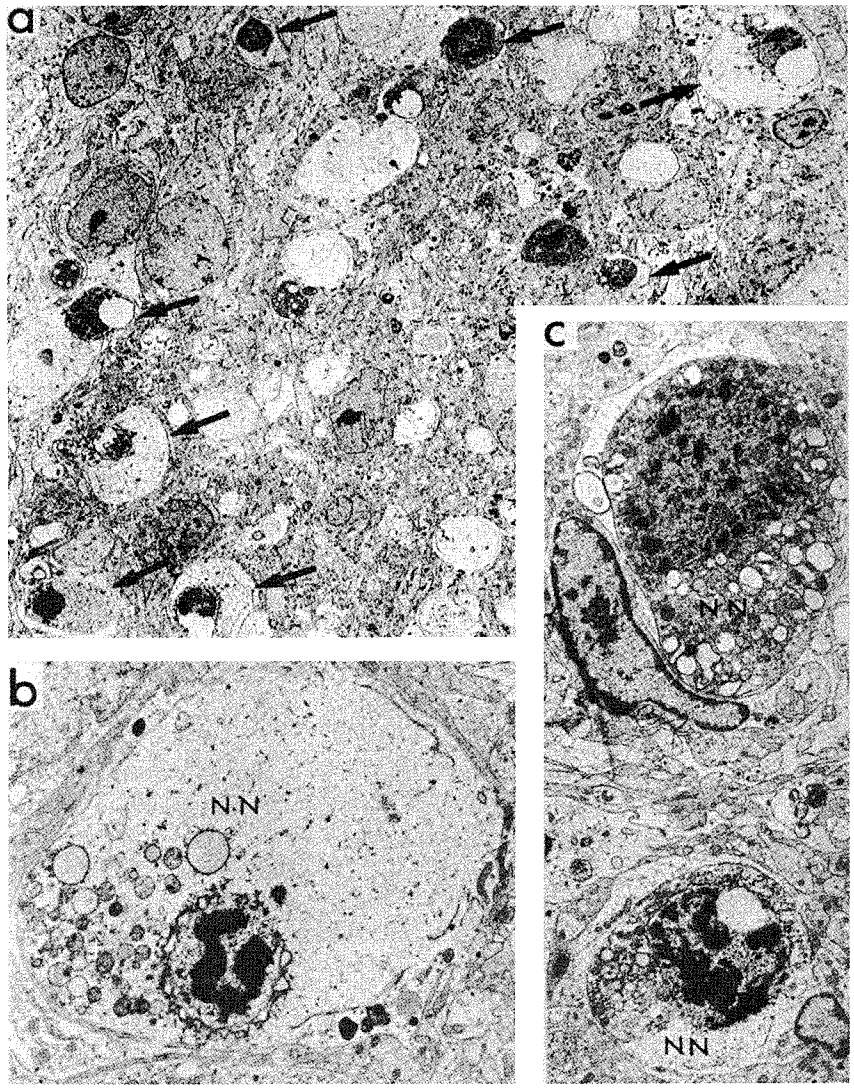

FIG. 13. **a:** Survey electron micrograph depicting a scene from an infant rhesus monkey that was tube fed a 2 g/kg dose of Glu mixed in milk 5 hr earlier. Note the numerous acutely necrotic neurons (*arrows*) throughout the field—a finding not readily explained in terms other than Glu neurotoxicity. Compare these acutely necrotic neurons and those in **b** and **c** of this figure with those from the Glu-treated infant mouse illustrated in Fig. 3b. × 950. **b,c**: Electron micrographs depicting acutely necrotic neurons (NN) from the AH region of an infant rhesus monkey that was tube fed a 1 g/kg dose of Glu mixed 5 hr earlier. In **c** there are two NN; the one above is being phagocytized by another cell having a flattened oblong nucleus. **b,** × 2,850; **c,** × 1,425.

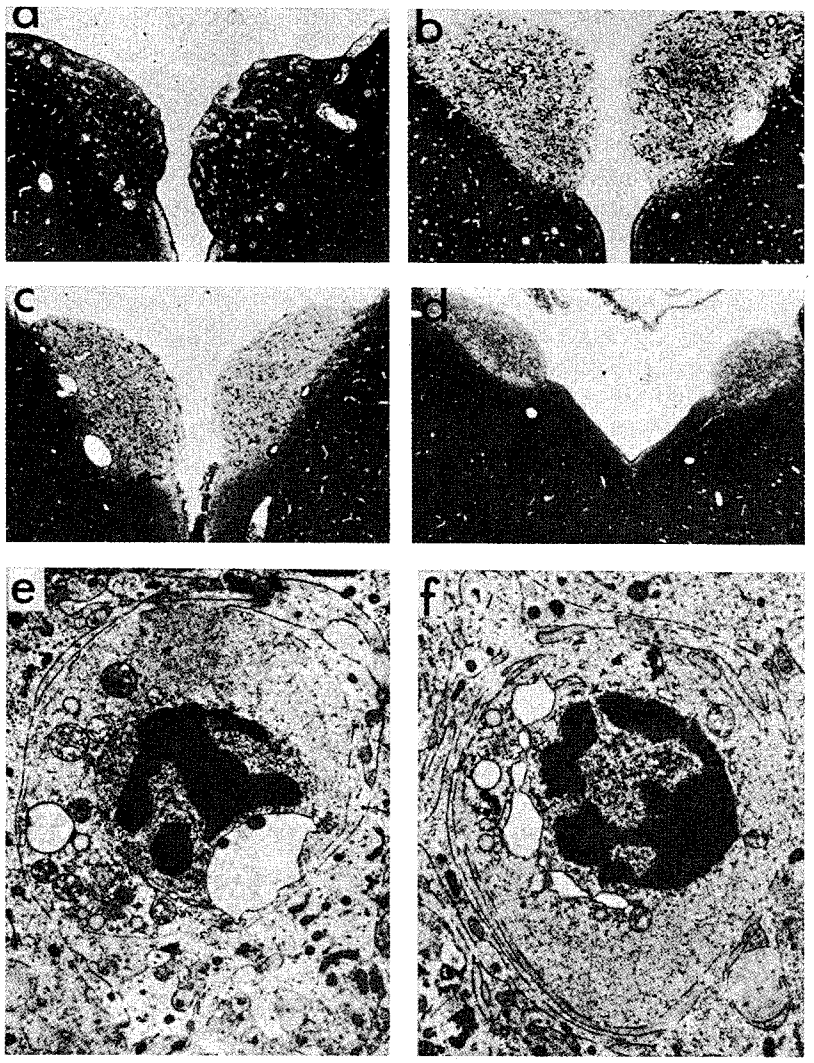

FIG. 14. **a–d:** Light micrographs illustrating the AP region from 4 rhesus monkeys, the 3 depicted in **b–d** being Glu treated and the 1 in **a** being an NaCl-treated control. The AP is a symmetrical organ in the primate, the margins of which are precisely outlined by the acute edema reaction in the Glu-treated monkeys (**b–d**), which gives the AP region an abnormally rarefied appearance not seen in the NaCl control **a**. The AP illustrated in **d** is from a pregnant rhesus monkey 5 hr after 2 g/kg Glu i.v. dose and the AP in **b** is from her fetus. The APs in **a** and **c** are from infants treated with NaCl (12 mmoles/kg) and Glu (16 mmoles/kg), respectively. **a–c,** × 27; **d,** × 18. **e,f:** Electron micrographs illustrating acutely necrotic neurons from the AP region of an infant rhesus monkey that was tube fed 2 g Glu/kg (12 mmoles/kg) mixed in milk 5 hr previously. × 1,800.

Committee concludes that the morphologic changes are real and are reproducible'' (98).

I agree with the tentative conclusion of the FASEB Committee (98) that Glu cannot be considered safe for use in infant or junior foods. If the amounts of Glu added to foods ingested by immature humans were in the range of those found naturally in human milk, no safety issue would have arisen. However, it is quite predictable that the concentrations used for food flavoring will always be excessive compared to concentrations in human milk because no flavor effect is achieved from Glu in the concentration range found in milk. For example, to achieve the desired flavoring effect, baby food manufacturers have used Glu in levels up to 0.6% (> 750 mg Glu per 4.5-oz jar of strained baby food) (50). Since human breast milk contains free Glu in the range of 30 to 35 mg/150 ml, one jar of the above baby food would contain 20 to 25 times more free Glu than is found in one feeding of human milk and would provide a human infant with more than 125 mg Glu/kg body weight, which is 25% of the oral load (500 mg/kg) known to destroy hypothalamic neurons in infant animal brain (70,106). Although blood-brain barriers protect most central neurons from Glu, it must be recognized that (a) certain brain regions lack such protection; (b) it requires only a transient increase in blood Glu levels for neurons in such regions to be destroyed; (c) mechanisms for preventing transient blood Glu elevations may be ineffective in youth or disease; (d) the addition of Glu to foods ingested by immature humans entails risk without benefit (meets no health or nutritional needs). The interested reader will find a more detailed discussion of safety considerations elsewhere (60,61).

CONCLUDING REMARKS

Considerable interest has developed recently in the use of excitotoxic amino acids as research tools for producing ''axon-sparing'' lesions in the central nervous system. I have given an historical account revealing how this application of excitotoxins in neurobiological research developed as an outgrowth of earlier inquiries into the neurotoxic properties of Glu, particularly molecular specificity inquiries that led to the realization that the neuroexcitatory amino acids in general—not just Glu—are axon-sparing neurotoxins. I have not covered in detail the most recent research developments pertaining to the use of the more potent excitotoxins as lesioning tools because an entire book concerning this theme, with contributions from all leading researchers in the field, was recently published (45). In this review I have placed proportionally greater emphasis on the uses of Glu and its excitotoxic analogs as neuroendocrine investigational agents, a topic that has been relatively slighted in the review literature. I have attempted to convey an impression of the extent and variety of information referable to neuroendocrine mechanisms that can be obtained from simple experiments involving systemic administration of excitotoxins, either alone or in combination with other pharmacological agents, to experimental animals. Excitotoxins penetrate select portions of the endocrine hypothalamus from blood and have the dual capacity of either stimulating or

destroying hypothalamic neurons, hence the versatility of serving alternatively as either provocative or ablative neuroendocrine research tools. Evidence presented here and elsewhere (59,81) documenting Glu-induced hypothalamic damage in rhesus monkeys suggests that the excitatory amino acids may be suitable agents for examining neuroendocrine regulatory mechanisms in primates as well as rodents. Risk factors associated with the use of Glu as an additive in foods ingested by human infants and children are briefly discussed.

ACKNOWLEDGMENTS

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