increases in serum prolactin concentrations in women than in men (*19*).

Our recent observation of a close correlation between the prolactin response to TRH and the concentration of pituitary TRH receptors (*5*) can probably offer a partial explanation for the results obtained. However, the almost complete reversal of the inhibitory effect of dopamine by E_2 clearly shows an important effect of E_2 on dopamine receptor action in the anterior pituitary gland. Since dopamine receptors are not only involved with prolactin secretion but appear to have a role in the modulation of mood and behavior (*20*), and in some diseases (*21*), the present data indicate that the control of prolactin secretion in anterior pituitary cells in culture could well be used as a model system for studies of the effects of peripheral hormones on dopaminergic action.

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Striatal Nondopaminergic Neurons: Possible Involvement in Feeding and Drinking Behavior

Abstract. *Intracaudate injections of kainic acid destroy striatal neurons containing acetylcholine and γ-aminobutyric acid but leave dopaminergic nerve terminals in this brain region intact. Rats injected with the drug are aphagic and adipsic, and have other behavioral abnormalities strikingly similar to those seen in animals with lesions in the dopaminergic nigrostriatal bundle.*

Brain dopaminergic neurons whose cell bodies originate in the pars compacta of the substantia nigra and whose terminals form synapses with cells in the neostriatum (*1*) play an important role in feeding and drinking behavior (*2*). However, little is known about the roles of other neurons in the caudate nucleus that may form synapses with this dopaminergic afferent system. The striatum contains relatively large concentrations of many putative neurotransmitter compounds—acetylcholine, γ-aminobutyric acid (GABA), serotonin, and substance P—in addition to dopamine (*3*); however, it is difficult to study these other types of striatal neurons separate from the contribution of the dopaminergic afferent system, because of their relatively close proximity to one another.

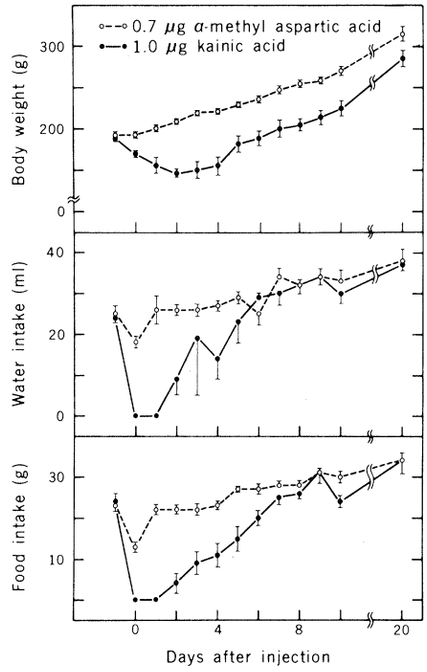

Fig. 1. Daily body weight changes and food and water intake after bilateral intracaudate injections of 1.0 μg of kainic acid or 0.7 μg of an iso-osmotic control solution of α-methylaspartic acid. All values are the means ± standard error of mean (S.E.M.) (N = 14).

Kainic acid (2-carboxyl-3-carboxymethyl-4-isopropenylpyrrolidine), a rigid cyclic analog of glutamic acid, is a powerful neuroexcitant when it is iontophoretically applied to brain or spinal cord neurons (*4*), and has neurotoxic effects on cells in the striatum after intracaudate injections of larger doses (*5*). Although these latter injections destroy striatal cholinergic or GABAergic nerves, nigrostriatal dopaminergic nerve terminals projecting into the caudate nucleus are apparently left intact.

Using intracaudate injections of kainic acid, we now report that rats become aphagic and adipsic after the treatment, and also have other behavioral abnormalities and permanent feeding and drinking regulatory impairments previously seen only in animals with electrolytic (*6*) or chemical (*2*) lesions of the nigrostriatal bundle.

Male albino rats (175 to 200 g; Charles River Laboratories, Wilmington, Mass.) had free access to food (Purina rat chow) and water, and were housed under a constant light-dark cycle (12:12 hours; lights on at 0800). Under Nembutal anesthesia, different groups of rats were stereotaxically injected with various doses of kainic acid or an iso-osmolar control solution (α-methylaspartic acid, 0.7 μg/μl

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dissolved in 0.9 percent saline). The unilateral or bilateral intracaudate drug injections were administered in a constant volume (1.0 μl in 1 minute) (*7*). Animals were housed individually in hanging wire cages with free access to food and water after surgery. Body weight and food and water intake were measured daily.

Other groups of animals were injected unilaterally with 1.0 μg of kainic acid or its diluent and were killed 2, 4, 7, or 60 days after injection. The caudate nuclei were rapidly dissected out, weighed, and assayed (*8*) for dopamine, GABA, or protein concentrations, and for choline acetyltransferase or glutamate decarboxylase, the brain enzymes catalyzing the synthesis of acetylcholine or GABA, respectively.

In contrast to rats receiving the control solution of α-methylaspartic acid, animals injected unilaterally or bilaterally with kainic acid became aphagic and adipsic, with the duration and severity of both behaviors related to the dose of the drug administered. Eighty-seven percent of the animals injected unilaterally with 1.0 μg of kainic acid were aphagic and adipsic on the first postinjection day, but ate food and drank water by the third day after injection. All animals receiving bilateral intracaudate injections of 1.0 μg of kainic acid were aphagic and adipsic on the first day after treatment, 33 percent ate food or drank water by 3 days after injection, and only 70 percent eventually recovered normal food and water intake patterns (the remaining animals died of inanition within the first 6 days after treatment) (Fig. 1). Although animals surviving the initial phase of aphagia and adipsia after this dose regained their preoperative body weights within 1 week, they weighed less than the control injected animals for as long as 60 days after injection (the longest time point measured). After bilateral injection with the highest doses of kainic acid (2.5 or 5.0 μg), rats never ate food or drank water spontaneously, and died from inanition within 1 week unless they were kept alive through intragastric force-feeding (*9*).

Animals injected bilaterally with kainic acid had crouched and humpback postures, walked gingerly on the tips of their toes, showed marked piloerection, and failed to groom themselves normally. These symptoms were evident only during the period of aphagia and adipsia, and were not caused by the inanition (normal animals deprived of food and water do now show these abnormalities). Similar postural and grooming abnormalities also occur in other animals with bilateral lesions of the nigrostriatal bundle (*9*). Animals with unilateral intracaudate injections of kainic acid (1.0 or 2.5 μg) showed pronounced contralateral rotational behavior for 1 to 2 days after treatment; however, this turning preference was absent in animals tested later (*10*).

Kainic acid injected unilaterally into the caudate nucleus markedly reduced the activities of the enzymes choline acetyltransferase and glutamate decarboxylase, but did not alter striatal dopamine concentrations (Fig. 2). In addition, GABA concentrations were reduced in the ipsilateral striatum and substantia nigra as long as 60 days after injection (GABA levels in the striatum and substantia nigra of kainic acid–treated animals were only 50 and 52 percent, respectively, of control values) (*11*). Taken together, these data replicate the neurochemical results of others (*5*) and suggest that kainic acid permanently damages striatal acetylcholine-containing intrinsic neurons and caudate GABA-containing nerves whose axons and terminals project to the substantia nigra (*12*). In contrast to these neurotoxic effects, intracaudate kainic acid injections apparently do not adversely affect dopaminergic nerve terminals in this brain region (*5*). These latter observations are critical, inasmuch as they suggest that the aphagia and adipsia seen in the kainic acid–treated rats most likely result from damage to striatal neurons, and are not simply the result of an action of the drug on the dopaminergic nigrostriatal afferent bundle.

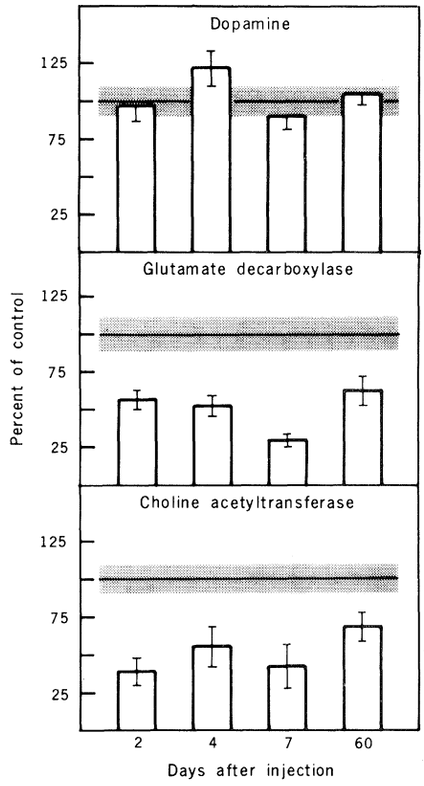

Fig. 2. Changes in dopamine concentration, glutamate decarboxylase, and choline acetyltransferase activities after intracaudate injection of kainic acid. Male albino rats were injected unilaterally with kainic acid (1.0 μg) or with a control solution of α-methylaspartic acid and were killed 2, 4, 7, or 60 days after treatment. Individual striata were dissected out and assayed for dopamine, glutamate decarboxylase, or choline acetyltransferase enzyme activity. The stippled area in each panel represents the values obtained from animals injected with the control solution and killed at the indicated times. No significant differences were found in any control injected animals, and they have been combined. All values are the mean percentage change from control values ± S.E.M. in kainic acid–treated animals. Control values were dopamine, 6.0 ± 0.6 μg per gram of tissue; glutamate decarboxylase activity, 9.6 ± 5.1 nm of CO_2 per milligram of protein per hour; and choline acetyltransferase activity, 102.8 ± 9.2 nm of acetylcholine per milligram of protein per hour.

Hence, intracaudate injections of kainic acid produce long-term feeding and drinking behavior deficits, motor response impairments, and postural abnormalities that are strikingly similar to those observed in animals with unilateral or bilateral lesions of the ascending dopaminergic nigrostriatal bundle. Since kainic acid appears to destroy only neurons whose cell bodies, but not axons or terminals, are in close proximity to the injection site (*5*), we conclude that the behavioral impairments seen in these animals are the result of damage to neurons that may normally be directly or indirectly innervated by nigrostriatal dopaminergic nerves. Similar injections of kainic acid also destroy substance P–containing neurons of the striatonigral pathway (*13*) as well as striatal cholinergic and GABAergic nerves. Thus, it is too early to speculate about the precise types of striatal neurotransmitter systems involved in the kainic acid–induced behavioral abnormalities. However, kainic acid should prove useful as a neuroanatomical tool that may allow us to completely map a brain neural circuit important for complex responses such as consummatory and motor behaviors (*13*).

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7. Different groups of animals ($N \geq 10$) were injected with α-methyl-aspartic acid or with 1.0 μg (unilaterally or bilaterally), 2.5, or 5.0 μg (bilaterally) of kainic acid. The stereotaxic coordinates for the injections in the midcaudate nucleus were A-P 8.6 mm and D-V 4.9 mm from the interaural line and M-L 2.7 mm from the midsagittal sinus suture. Some animals were perfused with 10 percent formalin, and 50-μm sections through the caudate nucleus were stained with cresyl violet. Kainic acid caused a marked cell body loss throughout this brain region, with the greatest damage occurring within a radius of 1.5 to 2.0 mm around the injection site.
8. The assay procedures are described as follows. Dopamine: J. T. Coyle and D. Henry, *J. Neurochem.* **21,** 63 (1971); GABA: J. T. Graham and M. H. Aprison, *Anal. Biochem.* **15,** 487 (1966); protein: O. H. Lowry, N. J. Rosenbrough, A. L. Farr, R. J. Randall, *J. Biol. Chem.* **193,** 265 (1951); choline acetyltransferase activity: B. Mannervik and B. Sorbo, *Biochem. Pharmacol.* **19,** 2509 (1970); and glutamate decarboxylase activity: J. R. Moskal and S. Basu, *Anal. Biochem.* **65,** 449 (1975).
9. No special postoperative recovery techniques were used. However, the period of aphagia and adipsia, and subsequent deaths in some groups of kainic acid–treated animals, were not merely the result of nonspecific drug toxicity. In other experiments we have maintained animals given high doses of kainic acid (2.5 or 5.0 μg, bilaterally) by force-feeding them high-calorie liquid diets. These animals eventually recover spontaneous feeding and drinking; however, they continue to weigh less than control animals, and they do not respond normally by increasing water intake following challenges with hypertonic saline injections (D. C. Pettibone, N. Kaufman, L. D. Lytle, in preparation). Rats similarly recovered from bilateral chemical (*2*) or electrical (*6*) lesions of the nigrostriatal bundle show other permanent feeding and drinking regulatory deficits, including impaired responses to glucoprivic or osmotic challenges, finicky responses to changes in diet palatability, and blunted responses to anorexic doses of amphetamine. Although kainic acid–treated animals share some of these deficits, it is too early to tell whether they are identical in all respects to those seen in animals with nigrostriatal lesions.
10. Animals with unilateral intracaudate injections of kainic acid (3.0 μg) show marked contralateral rotational behavior when tested 1 day after injection (33 ± 6 rotations per 15 minutes in kainic acid–treated animals compared to 3 ± 1 rotations in those treated with α-methyl-aspartic acid). This contralateral rotational preference gradually disappears, and is not apparent by 10 days after treatment. However, animals given unilateral injections of kainic acid show a strong ipsilateral rotational preference when challenged with *d*-amphetamine sulfate 10 days after kainic acid injection [25 ± 4 ipsilateral rotations per 15 minutes in kainic acid–treated animals compared to 9 ± 4 rotations in control injected rats ($P < .01$) (D. J. Pettibone, N. Kaufman, L. D. Lytle, in preparation). This drug-induced turning behavior is similar to that seen in animals with unilateral nigrostriatal lesions [U. Ungerstedt, *Acta Physiol. Scand. Suppl.* **367,** 49 (1971)].
11. Some evidence indicates that animals treated with doses of kainic acid large enough (> 1.0 μg) to reduce the activities of choline acetyltransferase and glutamate decarboxylase by more than 70 percent do not recover spontaneous eating and drinking unless force-fed with liquid diets.
12. The evidence that striatal cholinergic neurons are intrinsic is convincing [for example, E. G. McGeer, P. L. McGeer, D. S. Grewaal, V. K. Singh, *J. Pharmacol.* **2,** 143 (1975); F. Fonnum, *Brain Res.* **62,** 497 (1973); I. J. Bak, W. B. Choi, R. Hassler, *Adv. Neurol.* **9,** 13 (1975)] whereas support for the hypothesis that these cells form synapses in the caudate nucleus with dopamine nigrostriatal afferent nerves is largely indirect but compelling [P. M. Groves, C. J. Wilson, S. J. Young, G. V. Rebec, *Science* **190,** 522 (1975); V. H. Sethy and M. H. Van Woert, *Nature (London)* **251,** 529 (1974); D. L. Cheny, E. Costa, G. Kacagni, M. Trabucci, *Br. J. Pharmacol.* **52,** 427 (1974); P. G. Guyenet, Y. Agid, F. Javoy, J. C. Beaujouan, J. C. Rossier, J. Glowinski, *Brain Res.* **84,** 227 (1975)]. The evidence that GABA-containing perikarya are localized in the caudate-putamen or the globus pallidus is controversial [for example, P. L. McGeer and E. G. McGeer, in *GABA in Nervous System Function*, E. Roberts, T. N. Chase, D. B. Tower, Eds. (Raven, New York, 1976), pp. 487–495; J. S. Kim, I. J. Bak, R. Hassler, Y. Okada, *Exp. Brain Res.* **14,** 95 (1971)]; however, it is generally agreed that an inhibitory pathway to the substantia nigra is GABAergic [Y. Okada, in *GABA in Nervous System Function*, E. Roberts, T. N. Chase, D. B. Tower, Eds. (Raven, New York, 1976), pp. 235–243; M. Yoshida and W. Precht, *Brain Res.* **32,** 225 (1971); W. Precht and M. Yoshida, *ibid.*, p. 229].
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Receptors for Glucocorticoids in the Lens Epithelium of the Calf

Abstract. *The calf lens epithelium contains a specific cytoplasmic receptor for glucocorticoids. This binding protein has a high affinity for dexamethasone (average dissociation constant, 8×10^{-9} mole per liter), a low capacity (average, 550 femtomoles per milligram of protein), extreme heat sensitivity, and exhibits a pattern of competition similar to that of glucocorticoid receptors in other tissues. This provides direct biochemical evidence that these tissues may function as a target organ for glucocorticoids.*

Glucocorticoids have been shown to induce posterior subcapsular cataract in man following both topical and systemic administration (*1*). It is not known, however, whether the steroid hormones produce this effect by a direct action on lens tissue or through some secondary alteration of metabolism at another site. Since the primary action of a steroid hormone is its binding to a cytoplasmic receptor protein, we have initiated a search for glucocorticoid receptors in lens tissue. We report here on the presence of a glucocorticoid receptor in calf lens epithelium, which provides direct biochemical evidence that these cells may function as a target organ for glucocorticoids.

Calf lens was used since large amounts of tissue were readily available (*2*). For each experiment we used extracts prepared from 20 to 50 anterior capsules with adhering epithelial cells (*3*). The

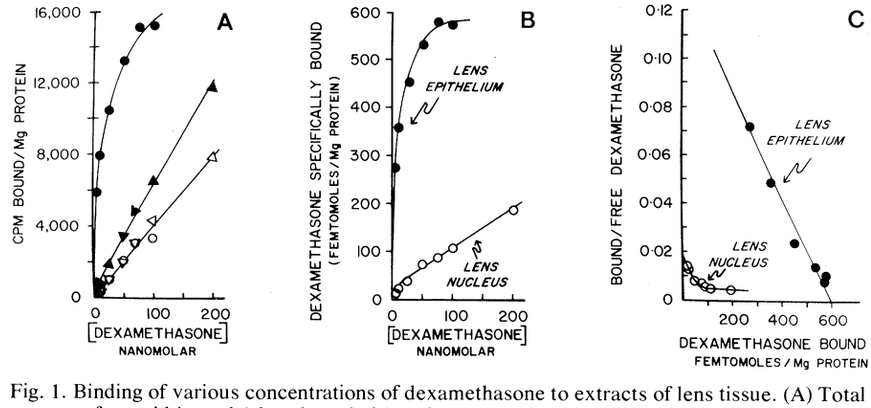

Fig. 1. Binding of various concentrations of dexamethasone to extracts of lens tissue. (A) Total amount of steroid bound (closed symbols) and amount of nonspecifically bound steroid (open symbols) for extracts from the lens epithelium (circles) and the lens nucleus (triangles). (B) Nonspecifically bound steroid has been subtracted from the total, and the specifically bound steroid is expressed in femtomoles per milligram of protein in the extracts. (C) Scatchard plot of the specifically bound dexamethasone. The equilibrium constant for the dissociation of the bound steroid, K_D, calculated from the slope, is $6 \times 10^{-9} M$. The protein concentrations in the extracts were: lens nucleus, 4.7 mg/ml, and lens epithelium, 1.26 mg/ml.

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