Table 1. Effect of the various prostaglandins on the MAO activity in rat liver utilizing tyramine and serotonin as substrates

	Tyramine (Sp. Act. ± S.E.)	Serotonin (Sp. Act. ± S.E.)
Control (7)	10.68 ± 0.26	14.19 ± 0.24
PGA_1 (7)	13.63 ± 0.16	22.65 ± 0.31
PGE_2 (7)	17.37 ± 0.30	22.11 ± 0.31
$PGF_{2\alpha}$ (7)	15.59 ± 0.32	23.14 ± 0.22

The numbers in brackets indicate the number of experiments conducted in each case. $P < 0.001$ in all the cases.

Table 2. Effect of the various prostaglandins on the MAO activity in rat brain utilizing tyramine and serotonin as substrates

	Tyramine (Sp. Act. ± S.E.)	Serotonin (Sp. Act. ± S.E.)
Control (7)	23.27 ± 0.19	37.40 ± 0.61
PGA_1 (7)	26.82 ± 0.41	56.87 ± 0.64
PGE_2 (7)	33.42 ± 0.27	60.05 ± 0.63
$PGF_{2\alpha}$ (7)	37.40 ± 0.07	52.19 ± 0.41

The numbers in brackets indicate the number of experiments conducted in each case. $P < 0.001$ in all the cases.

PGA_1, 60 per cent with PGE_2 and 40 per cent with $PGF_{2\alpha}$.

The monoamine oxidase activity was expressed in terms of specific activity, i.e. μg substrate utilized/mg protein. It is clear from our results that there is an increase in the activity of MAO in liver and brain on subcutaneous injections of prostaglandins A_1, E_2 and $F_{2\alpha}$. This increase in enzyme activity was observed with respect to serotonin as well as tyramine as substrates, thereby implicating a depletion of these amines in their storage depots, viz., tissues like liver and brain. This would lead to a decrease in the circulating level of these amines. The vasodilatory property of prostaglandins has been well established [10–13]. This vascular effect may be due to a decreased level of amines. Our results suggest that prostaglandins-induced headache, therefore, may be due to a depletion of amines which is brought about by an increase in the activity of monoamine oxidase.

In summary, prostaglandins are vasoactive substances, known to produce vasodilation. Intravenously administered PGE_1 and PGE_2 produce migraine headache. Prostaglandin was injected subcutaneously (150 μg in 0.3 ml per injection) at 1100 and 2300 hr everyday for 10 days. The MAO activity was measured in liver and brain using serotonin as well as tyramine as substrates. The increase in activity was significant with both the substrates in both the tissues, viz., liver and brain. The prostaglandins tested were PGA_1, PGE_2 and $PGF_{2\alpha}$ and the increased activity was observed in all the cases. Our results suggest that the depletion of amines produced by an increase in the activity of MAO may account for the headache induced by prostaglandins.

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CSIR Junior Research Fellow, Univ. Dept. of Biochemistry, Nagpur, University, Nagpur, India — VENKATARAMAN VIJAYALAKSHMI

UGC Junior Research Fellow, Univ. Dept. of Biochemistry, Nagpur University, Nagpur, India — JAYASHREE V. LELE

Lecturer in Biochemistry, Univ. Dept. of Biochemistry, Nagpur University, Nagpur, India — HATIM F. DAGINAWALA

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Pharmacological augmentation of acetylcholine levels in kainate-lesioned rat striatum

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Injection of kainic acid (KA), a conformationally restricted analogue of glutamic acid [1], into the rat striatum produces a complete degeneration of neurons, the cell bodies of which are within a 1.5 mm radius of the injection site, but spares axons from extrinsic neurons [2]. The striatal kainate lesion results in a marked reduction in pre-synaptic markers for cholinergic neurons in the striatum, including the activity of chlorine acetyltransferase, the levels of endogenous acetylcholine (ACh), and the activity of the synaptosomal high affinity uptake process for choline; the pre-synaptic

markers for the GABAergic* neurons are similarly reduced [2, 3]. In contrast, pre-synaptic markers for the dopaminergic and serotonergic projections to the striatum are not reduced [3, 4] and the cortico-striate fiber bundles remain intact [5]. The degeneration of the intrinsic striatal neurons is followed by an intense gliotic reaction [5]. The neurochemical and histologic sequelae of the striatal kainate lesion resemble closely the pathologic changes that occur in the striata and substantia nigra of patients affected with Huntington's disease [6–9] and, thus, provide a useful animal model for this human neurodegenerative disorder [2, 10, 11].

Clinical pharmacologic studies suggest that the cholinergic deficits in the striatum in Huntington's disease may play an important role in the pathophysiology of the movement disorder [6, 8, 11]. Based upon the success in alleviating symptoms of Parkinson's disease by augmenting dopaminergic function [12], it has been hypothesized that pharmacotherapy oriented toward augmenting cholinergic function may have beneficial effects on the movement disorder in Huntington's disease. Treatment with physostigmine, a centrally active inhibitor of acetylcholinesterase which elevates brain ACh levels, has been reported to reduce dyskinesia in some, but not all, cases [13, 14]. Similarly, systemic administration of choline chloride, which increases the brain levels of acetylcholine in experimental animals [15, 16], reduces symptoms of Huntington's disease in some, but not all, patients [14, 17, 18].

Evidence for the possible efficacy of cholinoceptive agents in altering striatal ACh has been derived exclusively from animals with an intact nigro-striatal circuit. However, degeneration of striatal neurons and the subsequent gliotic reaction in the area may alter considerably the disposition of drugs and neurotransmitter precursors; hence, extrapolation of results obtained in intact animals to this human degenerative disorder is questionable. Accordingly, to provide additional information regarding the potential therapeutic value of pharmacologic treatment in Huntington's disease, we have investigated the ability of systemically administered choline chloride and physostigmine to elevate ACh levels in the kainate-lesioned rat striatum.

*GABA = γ-aminobutyric acid.

Stereotaxic injection of kainic acid. Male, Sprague–Dawley rats (160—180 g) were anesthetized with Equithesin (0.65 ml, i.p.; Jensen-Salisbury Labs, Kansas City, MO) and positioned in a David Kopf small animal stereotaxic apparatus. A 0.3-mm cannula, attached to a 10-μl Hamilton syringe, was inserted through a burr hole in the calvarium; coordinates for the striatal injection were anterior to the bregma-0.8 mm, lateral-2.6 mm and ventral-4.8 mm. Kainic acid (Sigma Chemical Co., St. Louis, MO; Lot 105C-0064), 14 nmoles in a volume of 1 μl of artificial cerebral spinal fluid (CSF) buffered to pH 7.4, was infused into the corpus striatum over 40 sec. The cannula was then carefully removed, the burr hole was filled with dental cement, and the scalp was apposed with sutures.

Systemic drug treatments. Four days after placement of the kainate lesion, the lesioned and age-matched control rats received intraperitoneal injections of drugs dissolved in distilled water (1 ml/kg body wt.): choline chloride (Sigma Chemical Co.), 100 mg/kg, physostigmine sulfate (K & K Laboratories, Milwaukee, WI), 1 mg/kg, or an equal volume of vehicle.

Tissue preparation. At various times after drug treatment, the rats were killed by microwave radiation (3 sec; 1300 W; 2450 MHz) focused on the head in an oven adapted by Medical Engineering Consultants (Lexington, MA). The head of the caudate-putamen (striatum) was dissected and stored in a liquid nitrogen freezer until time of assay. Previous experiments had indicated that storage under these conditions does not affect neurotransmitter levels

Neurontransmitter assays. For measurements of endogenous neurotransmitters, the striata were homogenized in 20 vol. of 1 N formic acid–acetone (15:85) and centrifuged at 10,000 *g* for 15 min to remove protein. The levels of ACh and choline in the extracts were measured in 10-μl aliquots (0.5 mg tissue) by the radiometric enzymatic assay of Goldberg and McCaman [19]. Ten-μl aliquots of the extract were evaporated in microtubes in a vacuum centrifuge and assayed for GABA by the enzymatic fluorometric assay of Graham and Aprison [20]. Absolute levels of neurotransmitters were calculated on the basis of internal standards measured in duplicate samples for all treatment conditions, to preclude interference of drugs in the assay; no significant interference could be detected.

Table 1. Effects of choline chloride and physostigmine on ACh, choline and GABA in rat stratum*

	Treatment	Striatum		
		Control	Contralateral	KA-lesioned
ACh (pmoles/mg)	Vehicle	67 ± 6 (17)	58 ± 4 (19)	23 ± 2† (21)
	Choline chloride	78 ± 10 (11)	75 ± 7 (10)	45 ± 6†, ‡ (11)
	Physostigmine	110 ± 5†,‡ (7)	110 ± 12†,‡ (8)	44 ± 6†,‡ (11)
Choline (pmoles/mg)	Vehicle	58 ± 5 (13)	73 ± 6 (18)	89 ± 9† (17)
	Choline chloride	104 ± 16 (9)	105 ± 16 (7)	200 ± 54†,‡ (11)
	Physostigmine	56 ± 6 (8)	70 ± 12 (11)	67 ± 4 (8)
GABA (nmoles/mg)	Vehicle	2.3 ± 0.1 (18)	2.4 ± 0.1 (16)	1.1 ± 0.1† (20)
	Choline Chloride	2.4 ± 0.1 (10)	2.5 ± 0.1 (10)	1.7 ± 0.2†,‡ (10)
	Physostigmine	2.6 ± 0.3 (7)	2.4 ± 0.1 (8)	0.9 ± 0.1† (6)

*Each value is the mean ± S. E. M. for the number of striata indicated in parentheses. Choline chloride (100 mg/kg; 40 min) or physostigmine (1 mg/kg; 30 min) was injected i.p. into rats that had received a stereotaxic injection of 14 nmoles KA in the left striatum 4 days previously.

†$P < 0.05$, with respect to striata from unlesioned, vehicle-injected rats.

‡$P < 0.05$, with respect to striata from vehicle-injected rats, comparison made within vertical columns.

Statistical analysis. Statistically significant differences among the mean levels of neurotransmitters and interactions between lesion and systemic drug treatment effects were determined by two-way analysis of variance in a factorial design [21, SPSS Analysis of Variance and Covariance: Sub-program ANOVA]. Differences between individual mean neurotransmitter levels were assessed by Duncan's new multiple-range test [22]. The 5 per cent probability level was the criterion used for all significance statements.

Effect of striatal kainate lesion on levels of ACh, choline and GABA. The levels of ACh, choline and GABA in striata from control and KA-lesioned rats, after intraperitoneal injection of drug vechile, are shown in Table 1. While the ACh levels in striata contralateral to the KA lesion do not differ significantly from control, levels in lesioned striata are reduced by 65 per cent. The levels of endogenous choline in striata contralateral to the lesion are not significantly different from control whereas lesioned striata exhibit a significant elevation in choline. The level of endogenous GABA is reduced in lesioned striata by 52 per cent whereas the level in contralateral striata is not significantly different from control.

Effects of choline cholide on striatal ACh, choline and GABA. Treatment with choline chloride (100 mg/kg) 40 min prior to sacrifice does not significantly raise ACh in control striata or those contralateral to the kainate lesion, but it doubles the level of the neurotransmitter in the KA-lesioned striatum (Table 1). Similarly, increases in the levels of endogenous choline in control (79 per cent) and contralateral (43 per cent) striata are less dramatic than the increases seen in KA-injected striata (124 per cent). Choline treatment does not significantly alter GABA levels in the control or contralateral striata, but a significant 50 per cent increase in endogenous GABA is noted in the KA-lesioned striatum. In additional experiments, the effects of treatment with 200 mg/kg of choline were examined. The KA-lesioned rats are peculiarly vulnerable to this higher dose, with a 50 per cent rate of mortality, whereas unlesioned animals are not so affected. While the higher dose of choline results in significant elevation in the levels of endogneous ACh in control (92 ± 5 pmoles/mg) and contralateral (100 ± 16 pmoles/mg) striata, it does not produce a further increase in ACh levels in the KA-lesioned striatum (46 ± 8 pmoles/mg). The high dose of choline does not alter significantly GABA levels in control and contralateral striata; however, it produces a 70 per cent increase in the KA-lesioned striatum. The effects of choline are transient in the KA-lesioned striatum with ACh levels returning to lesioned-control values by 60 min after injection (Fig. 1).

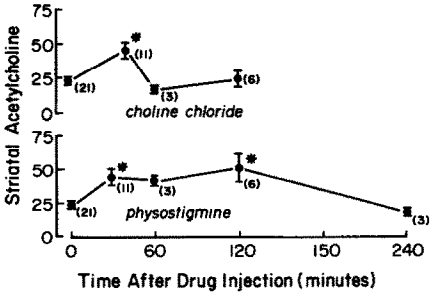

Fig. 1. Time course of drug effects on ACh (pmoles/mg) in KA-lesioned striata. Each value represents the mean ± S. E. for the number of striata indicated in parentheses. Drugs were injected i.p. in rats that had received a stereotaxic injection of 14 moles KA in the left striatum 4 days previously. The asterisk indicates $P < 0.05$ in respect to ACh level prior to systemic drug treatment.

Effects of physostigmine on striatal ACh, choline and GABA. Treatment of the rats with 1 mg/kg of physostigmine 30 min prior to death results in comparable increases, of approximately 80 per cent, in the levels of ACh in the control, contralateral and lesioned striata (Table 1). The acetylcholinesterase inhibitor does not affect striatal choline or GABA. The magnitude of the effect of physostigmine is similar to that of choline chloride but more prolonged; ACh levels are elevated by 88 per cent at 30 min, 68 per cent at 60 min, 115 per cent at 120 min, but have returned to pretreatment levels by 240 min, after injection (Fig. 1).

The results of this investigation demonstrate that systemic treatments with choline chloride or physostigmine elevate ACh in the KA-lesioned rat striatum. Since the most extensive striatal lesion with KA (25 nmoles) leaves the rat striatum totally devoid of intrinsic neurons but reduces pre-synaptic cholinergic markers by no more than 80 per cent [3], it appears that the striatum receives some cholinergic innervation from extrinsic neurons. Recently, a cholinergic projection to the head of the caudate nucleus from cell bodies in the anterior thalamus has been described [23]. A consistent 20 per cent reduction in choline acetyltransferase activity, with no changes in dopaminergic or GABAergic markers, occurs in the striatum after widespread lesion of the frontal, parietal and occipital cortex suggestive of a corticostriatal cholinergic projection [24]; presumably, the surviving cholinergic fibres in the KA-lesioned striatum are the locus of action of the cholinoceptive drugs examined in this study.

The control values for choline are moderately higher than [25] or equivalent to [26] those reported for microwave killed rats but well below those obtained in decapitated rats [27]. In spite of the marked loss of cholinergic innervation in the KA-lesioned striatum, the levels of endogenous choline are increased significantly by 50 per cent as compared to control. After lesion of the septohippocampal cholinergic pathway, the concentration of endogenous ACh in the hippocampus is reduced markedly, whereas choline is unaffected [28]. Thus, the bulk of the endogenous striatal choline exists in a compartment distinct from the cholinergic as well as other intrinsic neurons.

Inhibition of acetylcholinesterase, the enzyme that hydrolyzes acetycholine, by treatment with 1 mg/kg of physostigmine, is effective in doubling the levels of ACh in the kainate-lesioned striatum; however, physostigmine also doubles the levels of ACh in control and contralateral striata, indicating a lack of specificity of this agent. Administration of choline increases the levels of both choline and acetylcholine to a greater extent in the lesioned striatum than in the contralateral or control striata. The significantly elevated levels of choline in the lesioned striatum indicate an abnormality in disposition of choline under basal conditions. In addition, the marked disruption of striatal structure due to the neuronal degeneration may alter the permeability of the blood brain barrier, thus allowing greater access of plasma choline [5]. Interestingly, choline treatment also causes a significant elevation of GABA levels in the lesioned striatum. Since physostigmine, which produces a comparable increase in ACh levels, fails to alter GABA levels, this effect does not appear to be mediated directly by ACh.

These studies indicate that precursor loading or inhibition of degradation of ACh can, at least partially, correct ACh deficits in an animal model of Huntington's disease. What is particularly noteworthy is the differential response of the KA-lesioned striatum to choline, indicating that the normal striatum provides a poor model system for assessing the pharmacologic efficacy of these treatments. This conclusion is strengthened by a study of the GABAergic system in the KA-lesioned striatum, which exhibits an enhanced response to a GABA-T inhibitor when compared with normal striatum [29]. Nevertheless, clinical studies demonstrate inconsistent therapeutic effects of these cholinoceptive agents on the dyskinesia of Huntington's disease. This

variable effectiveness of treatments that may augment cholinergic function must be considered in the light of the multiple neuronal deficits, including GABAergic and substance P pathways [30], involved in the pathophysiology of the extrapyramidal symptons of Huntington's disease. Possibly, those individuals who prove unresponsive to cholinoceptive agents alone may respond to a combined treatment program directed at enhancing both cholinergic and GABAergic function.

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Departments of Pharmacology and Experimental Therapeutics, and Psychiatry and the Behaviorual Sciences, EDYTHE D. LONDON
The Johns Hopkins University School of Medicine, Baltimore, MD 21205, U.S.A. JOSEPH T. COYLE*

*Address reprint requests to : Joseph T. Coyle, M.D., Department of Pharmacology, John Hopkins University School of Medicine, 725 North Wolfe St., Baltimore, MD 21205.

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Effect of methylxanthines and elevated external potassium on high energy phosphate content in frog skeletal muscle*

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The active transport of sodium from frog skeletal muscle fibers can be stimulated by the methylxanthine, theophylline [1]. At a concentration of 2 mM the stimulation of sodium efflux appears reversible, whereas with higher doses of theophylline irreversible stimulation of sodium efflux, often associated with contractures, results. Since the irreversible stimulation of efflux was observed in sodium-free, strophanthidin-containing solutions, Hays *et al.* [1] speculated that this stimulation of sodium efflux might be a consequence of a significant decrease in the high energy phosphate content (PE) in these muscles. In order to test this idea, PE was determined in frog skeletal muscle after exposure to theophylline. Although few studies of the effects of theophylline on the functioning of frog skeletal muscle have been reported, another methylxanthine, caffeine, has been used extensively in frog skeletal muscle to study calcium movements and tension development. Therefore, for comparative purposes, PE was determined in muscles which had been exposed to levels of caffeine which in this preparation are either subthreshold or suprathreshold for contracture. In frog skeletal muscle, procaine blocks contractures elicited by suprathreshold levels of caffeine [2], blocks the increased oxygen consumption elicited by both sub- and suprathreshold levels of caffeine [3, 4] and blocks the alkalinization caused by subthreshold levels of both theophylline and caffeine [5]. Therefore, measurements were also made to determine if procaine could block the fall in PE seen in muscles exposed to subthreshold levels of theophylline and caffeine.

All measurements were made on whole sartorius muscles from the frog, *Rana pipiens*. Muscles were dissected, attached to stainless steel holders at resting length and allowed to

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