however, induced virtually complete resistance at concentrations that did not cause necrosis. This may mean that different mechanisms of resistance are involved or, because victorin at higher concentrations causes necrosis, it may be that actual necrosis is not required but only the initiation of a process which leads to it.

Although victorin and Victoria blight have served as a model system for extensive investigations of pathogenesis and the nature of disease resistance in oats (*1*, *11*), this is the first report of an effect of this pathotoxin on a plant which is not a member of the grass family. Failure to detect such effects in earlier investigations can be attributed in part to the highly selective nature of victorin; the 100 unit/ml required to injure bean leaves is 100,000 times higher than the 0.001 unit/ml which causes 50 percent inhibition of root growth in susceptible oats (*6*). Low sensitivity of the very young seedlings used in previous attempts to demonstrate effects of victorin on plants other than susceptible oats (*6*) may also have been a factor.

The extreme instability of purified preparations of victorin makes an accurate estimate of its potency impossible. However, the refined preparation used in this study contained, on a dry weight basis, 1 mg of solids per milliliter. Disease resistance was induced by a thousand-fold dilution of this preparation or at a concentration of solids of 1 $\mu$g/ml. Because our refined preparation was not pure, the actual potency must be much higher. This evidence of high potency, even to resistant tissues, and the fact that victorin strongly inhibits auxin-induced cell elongation (*12*) suggest that this pathotoxin has great potential for investigation of physiological processes in plants. In particular, victorin should provide a useful model system to study nonspecific defense mechanisms in higher plants.

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## m-Hydroxyphenylacetic Acid Formation from L-Dopa in Man: Suppression by Neomycin

Abstract. *The increased excretion of* m-*hydroxyphenylacetic acid in the urine of patients with parkinsonism being treated with* L-*dopa was reduced by gut sterilization with neomycin. The* p-*dehydroxylation step is thus brought about solely by the action of gut flora; the pathway is unlikely to be involved in the events within the brain leading to the therapeutic benefit effected by* L-*dopa.*

A substantial proportion of patients with parkinsonism obtain more therapeutic benefit from L-dopa (dihydroxyphenylalanine) than from any drug previously available (*1*). While it has been assumed that clinical improvement stems from dopamine generation within the central nervous system, Calne *et al.* (*2*) have pointed out that such a chemical transformation is likely to be rapid (*3*) whereas the time course of the therapeutic response to the drug is slow (*1*). The possibility cannot therefore be ruled out that the clinical effect derives not from dopamine replacement but from the buildup of some minor metabolite unconnected with the main route of dopa degradation. Therefore we charted minor pathways of dopa metabolism revealed by the large doses of drug employed (up to 8 g/day).

The existence of one such pathway, terminating in an increased urinary output of *m*-hydroxyphenylacetic acid (*m*-HPAA), was noted during a trial of dopa in patients with parkinsonism (*2*). DeEds *et al.* (*4*), who made a similar observation after feeding DL-dopa to rabbits, considered that *m*-HPAA might derive from the *p*-dehydroxylation of an intermediate in the reaction sequence, 3,4-dihydroxyphenylacetic acid. However, the possibility that the transformation occurs at some other stage, perhaps by *p*-dehydroxylation of dopa itself, of dopamine, or even of dihydroxyphenylpyruvic acid, with the remaining metabolic steps taking place after absorption of the dehydroxylated product, cannot be ruled out. A human stool suspension can bring about *p*-dehydroxylation in vitro of a variety of phenolic acids (*5*). If *p*-dehydroxylation of the catechol moiety by gut flora (*6*) were an essential step in the production in vivo of *m*-HPAA from L-dopa in man, gut sterilization with neomycin might decrease the urinary output of *m*-HPAA.

Six patients with idiopathic parkinsonism, receiving their maximum tolerated oral dosage of L-dopa (Fig. 1), were given oral doses of neomycin (1 g) daily. L-Dopa metabolism is unlikely to differ in healthy subjects and subjects with parkinsonism (*2*). Urine samples were collected before and during day 3 of neomycin treatment. The *m*-HPAA was isolated from urine saturated with salt (3 ml diluted to 10 ml with 0.01*N* HCl) at *p*H 2.0 by extracting twice (25 ml) with ethyl acetate. Portions (20 and 25 ml, respectively, pooled) of the extracts were evaporated to dryness under vacuum at 40° to 50°C, and the methyl ester–trimethylsilyl ether derivative was prepared. This was separated from other phenolic acid derivatives by isothermal (190°C) gas chromatography (*7*) on a Pye Panchromatograph with a 210-cm 10 per-

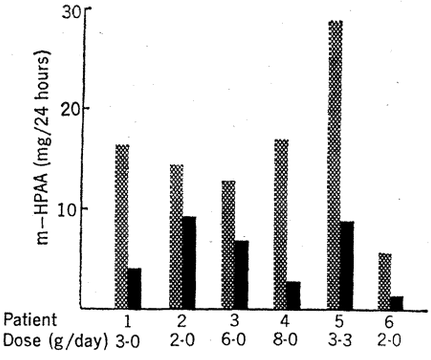


Fig. 1. Excretion of *m*-hydroxyphenylacetic acid (*m*-HPAA) before (hatched columns) and during (solid columns) day 3 of oral administration of neomycin (1 g/day). The subjects were six patients with parkinsonism being treated orally with L-dopa at the dosage shown.

cent SE52 column. The peak corresponding to *m*-HPAA was characterized by its methylene unit value and quantitated by comparing peak heights before and after the addition of the authentic acid as internal standard to duplicate urine samples.

Even on the comparatively low dosage scale of antibiotic employed, a significant ($P < .01$) decrease in *m*-HPAA output was detected (Fig. 1), from a mean ± S.E. of 16.0 ± 3.10 mg per 24 hours before neomycin to one of 5.6 ± 1.35 mg per 24 hours during its administration. Thus, an as yet unidentified intestinal microorganism or group of microorganisms sensitive to neomycin is apparently responsible for the formation of *m*-HPAA from L-dopa. Neomycin suppresses the excretion in human urine of a miscellaneous group of *m*-hydroxylated phenolic acids, presumably derived from dietary catechols (*8*). Similarly the increased excretion of *m*-hydroxylated acids which follows the oral administration of certain catecholic acids both in man (*9*) and the experimental animal (*10*) can be abolished by neomycin.

The *p*-dehydroxylation of L-dopa or one of its catechol derivatives apparently takes place by the action of gut flora within the gastrointestinal tract. The possibility is remote that this pathway is concerned in the train of events culminating in the clinical improvement observed during L-dopa treatment of parkinsonism; the question might be settled however by ascertaining whether a more prolonged trial of neomycin results in clinical deterioration. Such a trial might also indicate whether any of the side effects of L-dopa therapy are eliminated by gut sterilization and thus perhaps stem from the production of *m*-hydroxylated amines by gut flora.

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## Prostaglandins $E_1$ and $E_2$ Antagonize Norepinephrine Effects on Cerebellar Purkinje Cells: Microelectrophoretic Study

Abstract. *In microelectrophoretic experiments, prostaglandins $E_1$ and $E_2$ antagonize the reduction in discharge rate of cerebellar Purkinje cells produced by norepinephrine. Slowing of discharge evoked by 3′,5′-adenosine monophosphate or gamma aminobutyric acid is not antagonized. These data provide the first indication that endogenous prostaglandins may physiologically function to modulate central noradrenergic junctions.*

The prostaglandins are a class of endogenous, biologically active acidic lipids whose physiological functions have not yet been determined. Prostaglandin $E_1$ ($PGE_1$) modulates the target organ effectiveness of several hormones or neurohumors. It is thought to act in some cases by preventing activation of adenyl cyclase, the anabolic enzyme of 3′,5′-adenosine monophosphate (cyclic AMP) (*1*). This mechanism of action has been implicated in the antagonistic influence of $PGE_1$ on catecholamine-induced lipolysis (*2*), on the diuretic action of vasopressin on the toad bladder (*3*), and on the histamine- or gastrin-evoked increase in acid production by the gastric mucosa (*4*).

In the central nervous system there have been even fewer clues to the functional role of prostaglandins. However, their release from spinal cord (*5*), cerebral cortex (*6*), and cerebellum (*7*) has been recorded, as has their presence in the synaptosome fraction of the cerebral cortex (*8*). Furthermore, the discharge rate of single neurons in the cat brainstem can be affected by microelectrophoresis of the prostaglandins (*9*).

We have previously (*10*) shown that microelectrophoresis of norepinephrine (NE) reduces the spontaneous discharge of Purkinje cells in the rat cerebellum. Since this effect is also produced by microelectrophoretically administered cyclic AMP and since both the NE and cyclic AMP effects are potentiated by theophylline and aminophylline (inhibitors of the catabolism of cyclic AMP), we proposed that the response to NE may be mediated by activation of adenyl cyclase. We report here that microelectrophoretically administered prostaglandins antagonize the responses of Purkinje cells to NE.

Adult albino rats were anesthetized with chloral hydrate (350 mg/kg). Five-barreled micropipettes were used to record single units extracellularly, and to administer drugs to single neurons at the site of recording by microelectrophoresis. Previously described electrical methods prevented polarization of the electrode tip during drug ejection as well as undesirable diffusion of drugs from the micropipette (*11*). Solutions of $PGE_1$ (1 percent) or $PGE_2$ (0.65 percent) were prepared by suspending the acids in distilled water, subjecting the mixture to sonication, and titrating to *p*H 7.5 to 8 with 1*M* NaOH. Both $PGE_1$ and $PGE_2$ were ejected from the pipette barrels as anions.

Spontaneously active nerve cells were identified as Purkinje cells on the basis of the so-called "inactivation potentials" or "climbing-fiber responses" (*12*): high-frequency (300 to 500 per second) bursts of two to five spikes superimposed on a slow wave seen in capacitance-coupled recordings. In this study, neurons exhibiting such bursts also showed a rapid irregular rate of single spike spontaneous discharge (25 to 100 per second).

The direct effect of electrophoretically administered prostaglandins on the discharge frequency of Purkinje cells was somewhat variable (Table 1). However, $PGE_1$ had an unequivocal effect on 76 percent of the 33 units studied. Most Purkinje cells (64 percent) showed an increase in mean firing rate (Fig. 1A). Histograms of interspike intervals revealed that the elevation of mean rate produced by $PGE_1$ is primarily due to a reduction in the number and dura-