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.

M. SANDLER, F. KAROUM
C. R. J. RUTHVEN
*Bernhard Baron Memorial Research Laboratories* and *Institute of Obstetrics and Gynaecology, Queen Charlotte's Maternity Hospital, London, England*
D. B. CALNE
*Medical* and *Neurological Units, University College Hospital, London*

### References and Notes

1. D. B. Calne, G. M. Stern, D. R. Laurence, J. Sharkey, P. Armitage, *Lancet* **1969-I**, 744 (1969); G. C. Cotzias, P. S. Papavasiliou, R. Gellene, *N. Engl. J. Med.* **280**, 337 (1969).
2. D. B. Calne, F. Karoum, C. R. J. Ruthven, M. Sandler, *Brit. J. Pharmacol. Chemother.* **37**, 57 (1969).
3. A. Pletscher, G. Bartholini, R. Tissot, *Brain Res.* **4**, 106 (1967).
4. F. DeEds, A. N. Booth, F. T. Jones, *J. Biol. Chem.* **225**, 615 (1957).
5. A. N. Booth and R. T. Williams, *Biochem. J.* **88**, 66P (1963).
6. R. R. Scheline, *Acta Pharmacol. Toxicol.* **26**, 189 (1968).
7. F. Karoum, C. R. J. Ruthven, M. Sandler, *Clin. Chim. Acta* **20**, 427 (1968); F. Karoum, C. O. Anah, C. R. J. Ruthven, M. Sandler, *ibid.* **24**, 341 (1969).
8. A. M. Asatoor, M. J. Chamberlain, B. T. Emmerson, J. R. Johnson, A. J. Levi, M. D. Milne, *Clin. Sci. (London)* **33**, 111 (1967).
9. K. N. F. Shaw, M. Gutenstein, J. B. Jepson, *Proc. Int. Congr. Biochem.* **9**, 427 (1961); J. Dayman and J. B. Jepson, *Biochem. J.* **113**, 11P (1969).
10. J. C. Dacre and R. T. Williams, *J. Pharm. Pharmacol.* **20**, 610 (1968); J. C. Dacre, R. R. Scheline, R. T. Williams, *ibid.*, p. 619.
11. We thank the Smith Kline and French Foundation for a grant for gas chromatography equipment and the Wellcome Trust for support of D.B.C.

7 July 1969; revised 22 September 1969

## 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-

tion of the normally occurring pauses between periods of rapid irregular firing (Fig. 1B), with little change in the most probable interspike interval. The $PGE_2$ was more variable than the $PGE_1$ in its action on Purkinje cells (Table 1). It reduced the discharge rate of 42 percent of cells studied (Fig. 1A), while 38 percent were accelerated.

Despite the duality of their direct effects, microelectrophoretic administration of both $PGE_1$ and $PGE_2$ reproducibly antagonized the action of electrophoretically applied NE (Table 1 and Fig. 2). In these experiments, the minimum test dose (*13*) of NE which would produce complete cessation of single spike discharge was chosen. The antagonism could usually be demonstrated either by the microelectrophoretic administration of prostaglandin approximately 0.5 minute before and during the brief ejection of maximally effective doses of NE from another barrel of the same five-barrel pipette (Fig. 2B) or by prompt reversal of the NE-induced depression of discharge by subsequent microelectrophoretic administration of prostaglandin (Fig. 2A). Both types of blockade could be demonstrated in the same Purkinje cell. In 36 of 40 cells studied with either $PGE_1$ or $PGE_2$, there was antagonism of the NE response (Table 1). In 27 cells, the prostaglandin reproducibly and reversibly antagonized the norepinephrine depression by 40 percent or more.

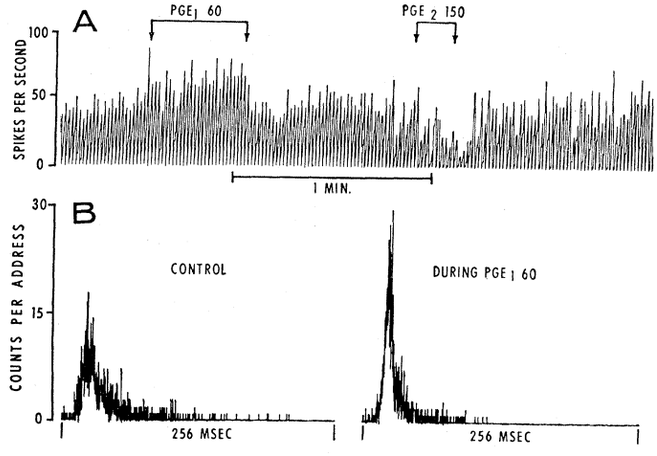
Table 1. Effects of microelectrophoretically applied $PGE_1$ and $PGE_2$ on Purkinje cells; 0, no effects; E, discharge rate elevated; R, discharge rate reduced; V, variable or slight antagonistic action; P, pronounced and repeateable antagonism.

| Agent | Direct action (No. cells) | | | Antagonism of NE response (No. cells) | | |
|---|---|---|---|---|---|---|
| | 0 | E | R | 0 | V | P |
| $PGE_1$ | 8 | 21 | 4 | 2 | 6 | 19 |
| $PGE_2$ | 5 | 9 | 10 | 2 | 3 | 8 |

Several lines of evidence indicate that the inhibition of NE responses by prostaglandin is due to antagonism, rather than to nonspecific Purkinje cell activation. First, blockade of NE action was frequently found with doses of prostaglandin which had no effect on discharge rate when ejected alone (Fig. 2B). Second, there were nine instances in which $PGE_1$ or $PGE_2$ reduced the spontaneous firing rate and also antagonized the NE depression. Finally, prostaglandin had no effect upon the depressant activities of either cyclic AMP (ten cells) or γ-aminobutyric acid (seven cells), as shown in Fig. 2, C and D.

Linoleic and linolenic acids were also tested in order to eliminate the possibility that NE antagonism might be due merely to a nonspecific action, such as the binding of cationic NE by the acidic lipids. However, neither of these fatty acid precursors of prostaglandin antagonized NE (ten cells).

Biochemical (*14*), histochemical (*15*), ultrastructural, and neuropharmacological (*10*) evidence suggests the existence of a noradrenergic inhibitory input to the Purkinje cell of the rat cerebellum. Thus, the acceleratory responses of Purkinje cells to the prostaglandins may represent disinhibition, that is, blockade of a tonic noradrenergic inhibition. Evi-



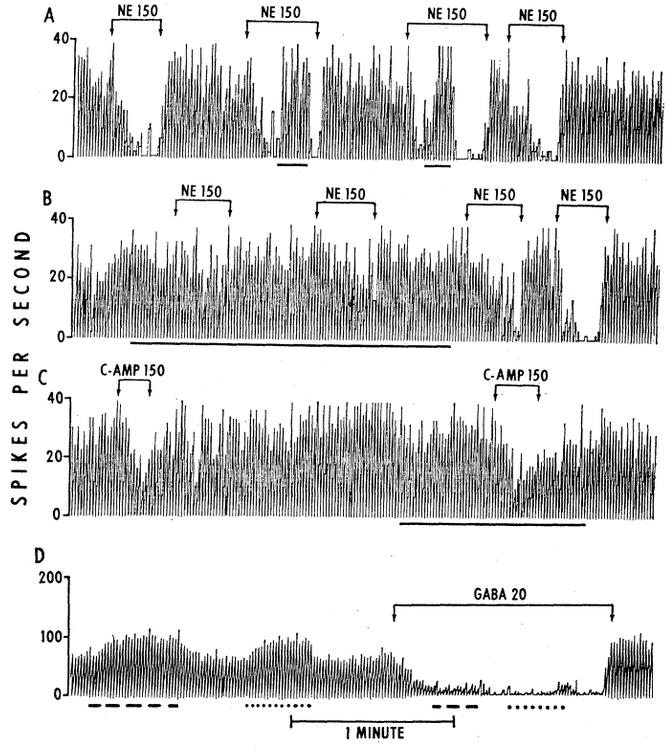




Fig. 1 (above). Effects of microelectrophoretic application of $PGE_1$ and $PGE_2$ on spontaneous Purkinje cell discharge. (A) Effects of drug application on mean discharge frequency. Duration of drug application indicated by arrows, numbers after each drug indicate ejection current in nanoamperes. (B) Interspike interval histograms of the same cell during the control period and during application of $PGE_1$: 1000 spikes and 0.25 msec address interval used for each histogram. The peak of the histogram represents the most probable interspike interval of single spike discharge. Note that the increase in mean discharge produced by $PGE_1$ is accompanied by a decrease in the population of long pauses (tail of the histogram) but little change in the most probable interspike interval (22 msec). Fig. 2 (right). Selective antagonism by prostaglandins of Purkinje cell responses to norepinephrine. A, B, and C represent consecutive records from the same cell. D illustrates another cell from a different preparation. Duration of NE, cyclic AMP, and γ-aminobutyric acid microelectrophoresis indicated by arrows. Numbers after each drug indicate ejection current in nanoamperes. Black lines beneath the records in A, B, and C represent microelectrophoresis of $PGE_1$, 80 na. The dashed and dotted lines beneath the record in D represents microelectrophoresis of $PGE_1$, 125 na and $PGE_2$, 125 na, respectively. Concentrations of prostaglandin which have no direct effect on mean discharge rate (B) antagonize the depressant effects of NE (A, B) but not cyclic AMP (C). Even doses of prostaglandin which directly increase mean discharge rates have no effect on the depressant effects of γ-aminobutyric acid (D).

dence supporting such a disinhibitory mechanism has been obtained from studies of rats treated with 6-hydroxydopamine. After intracisternal injection of this substance, there is a specific destruction of the NE-containing terminals, as well as a complete depletion of biochemically detectable NE, in the rat cerebellar cortex (*16*). Correlated with the absence of any presumed adrenergic inhibitory input in such animals, $PGE_1$ accelerated firing in only 7 percent of the 28 Purkinje cells examined, as opposed to 64 percent in control animals.

Further evidence favoring disinhibition by adrenergic blockade accrues from study of the interspike interval histogram. During the slowing produced by electrophoresis of NE, the action potentials occur at the same most probable interspike interval. However, the pauses between the periods of rapid firing are markedly augmented (*10*). The complementary change is seen during the acceleration of firing produced by electrophoretic application of prostaglandin; the most probable interspike interval is unchanged, but the normally occurring pauses in spontaneous discharge, represented by the "tail" of the histogram, are reduced in duration and number (Fig. 1B).

It is difficult to define the mechanism of the reductions in spontaneous discharge seen here and observed before (*9*) with prostaglandins. They may be produced by direct effects upon the Purkinje cells, by indirect actions on presynaptic elements, or by vascular changes. Whatever the mechanisms, they do not appear to involve adrenergic presynaptic elements (*17*), as the incidence of depression of discharge by prostaglandin in animals treated with 6-hydroxydopamine is not statistically different from that in normal animals.

The prostaglandin antagonism of NE responses in the cerebellum fortifies our hypothesis that NE effects are mediated through activation of adenyl cyclase (*10*). The prostaglandins and NE may influence Purkinje cells via a reciprocal interaction with adenyl cyclase, although other possibilities cannot be excluded without biochemical determinations. Purkinje cell slowing elicited by microelectrophoretic administration of aminophylline is also antagonized by prostaglandin (*18*). However, the failure of the prostaglandins to influence responses to cyclic AMP is significant, since cyclic AMP acts beyond this postulated site of prostaglandin action. This proposed mechanism for central neurons is quite similar to that for several effector systems in the peripheral nervous system (*2–4*). In view of the presence (*7*) and spontaneous release (*19*) of prostaglandin from the cerebellum, this lipid may indeed modulate cerebellar adrenergic junctions.

BARRY J. HOFFER, GEORGE R. SIGGINS
FLOYD E. BLOOM
*Laboratory of Neuropharmacology, Division of Special Mental Health Research, National Institute of Mental Health, St. Elizabeths Hospital, Washington, D.C. 20032*

**References and Notes**

1. S. Bergstrom, L. A. Carlson, J. R. Weeks, *Pharmacol. Rev.* **20**, 1 (1968).
2. R. W. Butcher and C. E. Baird, *J. Biol. Chem.* **243**, 1713 (1968); K. Stock, A. Aulich, E. Westerman, *Life Sci.* **7**, 113 (1968); E. W. Horton, *Physiol. Rev.* **49**, 112 (1969).
3. J. Orloff, J. S. Handler, S. Bergstrom, *Nature* **205**, 397 (1965).
4. A. Robert, J. E. Nezamis, J. P. Phillips, *Amer. J. Digest. Dis.* **12**, 1073 (1967); J. E. Shaw and P. W. Ramwell, *J. Physiol.* **195**, 34P (1968).
5. P. W. Ramwell, J. E. Shaw, R. Jessup, *Amer. J. Physiol.* **211**, 998 (1966).
6. P. W. Ramwell and J. E. Shaw, *Life Sci.* **2**, 419 (1963).
7. F. Coceani and L. S. Wolfe, *Can. J. Physiol. Pharmacol.* **43**, 445 (1965).
8. K. Katoaka, P. W. Ramwell, R. Jessup, *Science* **157**, 1187 (1967).
9. G. L. Avanzino, P. B. Bradley, J. H. Wolstencroft, *Brit. J. Pharmacol. Chemother.* **27**, 157 (1966).
10. G. R. Siggins, B. J. Hoffer, F. E. Bloom, *Science* **165**, 1018 (1969).
11. G. C. Salmoiraghi and F. F. Weight, *Anesthesiology* **28**, 54 (1967).
12. These responses are uniquely characteristic of the Purkinje cell in both cat [J. C. Eccles, R. Llinás, K. Sasaki, *J. Physiol.* **182**, 268 (1966)] and rat [D. J. Woodward, B. J. Hoffer, L. W. Lapham, *Exp. Neurol.* **23**, 120 (1969)].
13. In this context, the term "dose" is relative and will be used interchangeably with the intensity of ejection current. Serial ejections of NE or prostaglandin with the same current strength evoked reproducible responses. Biochemical studies done with Dr. N. Neff indicate that serial application of the same current into brain release similar quantities of NE.
14. L. L. Iversen and J. Glowinski, *Nature* **210**, 1006 (1966).
15. N. E. Andén, K. Fuxe, U. Ungerstedt, *Experientia* **23**, 838 (1967); T. Hökfelt and K. Fuxe, *Exp. Brain Res.* **9**, 63 (1969); G. Jonsson, K. Fuxe, B. Hamburger, T. Hökfelt, *Brain Res.* **13**, 190 (1969).
16. F. E. Bloom, A. Groppetti, A. Revuelta, E. Costa, Proc. Int. Congr. Pharmacol. 4th, Basel, July 1969.
17. P. Hedqvist, *Acta Physiol. Scand.* **75**, 511 (1969); P. Hedqvist and J. Brundin, *Life Sci.* **8**, 389 (1969).
18. B. J. Hoffer, G. R. Siggins, F. E. Bloom, unpublished observations.
19. S. W. Holmes and E. W. Horton, *J. Physiol.* **195**, 731 (1968).
20. We thank Dr. J. Pike and the Upjohn Company for supplying various prostaglandins and A. P. Oliver for technical assistance.

30 July 1969

## Hageman Factor (Factor XII) Deficiency in Marine Mammals

Abstract. *Hematologic and coagulation studies were conducted on Atlantic bottlenose dolphins and killer whales. Hematologic values were similar to those in man. These animals differed from other mammals in that the Hageman factor (factor XII) was absent and this absence caused marked prolongation of coagulation. Levels of factors VIII and V were high and those of VII and X were low compared with levels in man.*

Interest in marine mammals has increased greatly in recent years, but information on the normal physiology of these animals is fragmentary. Recently Puppione (*1*) noted a delayed initiation of blood clotting in samples obtained from members of the cetacean order of marine mammals. This order includes dolphins, porpoises, and whales. Stimulated by this report, we studied the blood coagulation and hematology in six trained cetaceans—one female and two male Atlantic bottlenose dolphins (*Tursiops truncatus*) and one female and two male killer whales (*Orcinus orca*).

Blood for coagulation studies was obtained in plastic syringes from veins on the ventral surface of the flukes of the unanesthetized animals. After separation of plasma or serum from cells, specimens were kept frozen at −20°C in glass and plastic tubes until the laboratory tests could be carried out. Coagulation determinations were performed by standard or previously described methods for studying man (*2*); fibrinolytic studies were done according to methods of Alkjaersig *et al.* (*3*).

Hematologic values are given in Table 1. The blood cells were morphologically similar to those seen in man. An occasional nucleated red blood cell was present. The percentage of eosinophils was strikingly greater in the three dolphins than in the whales and man, but we have no reason to suspect that this was pathologic since others (*4*, *5*) have reported similar findings. However, Ridgeway suggested that this might be an indication of universal parasitism in the dolphins. By human standards, the platelet counts were within normal limits in the whales and somewhat low in the dolphins.

The results of general coagulation