Evidence Against Prostaglandin E Having a Physiological Role in Acetylcholine Liberation from Auerbach's Plexus of Guinea-Pig Ileum

More than half a century ago OTTO LOEWI discovered that neurons (e.g., vagus nerve) communicated with effector organs (e.g., cardiac muscle) by a chemical substance, acetylcholine (ACh). Subsequently, HENRY DALE and his coworkers showed that ACh is not only the communicator in other neuroeffector organs (e.g., skeletal muscle) but also the communicator between neurons (e.g., sympathetic ganglia). These researches laid the foundation for understanding the chemical transmission of nerve impulses. Since the discovery that ACh is a neurotransmitter, the most complex role of ACh in mammalian central nervous system has been decoded based on the discovery that ACh-containing neurons maintain the neuronal integration of the states of vigilance (wakefulness) and paradoxical sleep (dreaming)[1-3].

Recently, two different groups of investigators[4,5] have suggested that prostaglandin E (PGE) has a role in ACh liberation. A closer examination, however, reveals a contradiction between the postulates presented by the two groups. According to WENNMALM and HEDQVIST[4], PGE regulates cholinergic transmission by a negative feedback; therefore, a lack of PGE should increase ACh output. According to EHRENPREIS et al.[5], however, a lack of PGE should block ACh output. Neither of the postulates were based on measurements of ACh output but, rather, on the end-organ effect of either exogenous PGE or PGE synthesis inhibitor. EHRENPREIS et al.[5] based their view on the observation that indomethacin (INDO), a PGE synthesis inhibitor, at a concentration of 45 μg/ml blocked field stimulated longitudinal muscle contractions of guinea-pig ileum. The study presented here was designed to investigate whether PGE does have a role in ACh liberation. To this end, the effects of INDO (15–45 μg/ml) on ACh output from Auerbach's plexus of guinea-pig ileum was investigated.

Table I. Effect of indomethacin on the spontaneous acetylcholine output from Auerbach's plexus of guinea-pig ileum

	Indomethacin concentration (μg/ml)			
	0	15	30	45
Animal group[b]	Acetylcholine output: (ng/g/min)[a]			
I(6)[c]	13.91 ± 3.46	13.92 ± 2.27		
II(6)	12.60 ± 0.73		12.80 ± 1.02	
III(6)	19.60 ± 3.70			16.62 ± 2.97

[a] Mean ± estimated standard error, equivalent to acetylcholine bromide. [b] Each animal provided only 1 pair of test strip: 2 different segments of ileum were used to prepare 2 longitudinal muscle strips with attached Auerbach's plexi; one strip acted as the control, the other was incubated in indomethacin. Tissues were incubated in the bath ± indomethacin for 30 min; the bath fluid was replaced with fluid containing physostigmine ± indomethacin and a second 30 min incubation was performed. At the end of this second incubation, this bath fluid was assayed for acetylcholine content (for detail see text). Unknown samples (0.1–0.2 ml) were bracketed between known ACh, which was dissolved in Tyrode's solution containing 2 μg/ml physostigmine to match control unknown samples and physostigmine + appropriate concentration of indomethacin to match indomethacin treated unknown samples. [c] Number of animals; to derive number of experiments multiply each number with 2, one half the number, controls.

Methods. Guinea-pigs, 275–450 g, were sacrificed by stunning, and the small intestine was disected and placed in Tyrode's solution, which was gassed with 95% O_2/5% CO_2. Two different segments of ileum were used to prepare 2 strips of longitudinal muscle with attached Auerbach's plexi[6], one strip acted as the control, the other was treated with INDO. Each muscle strip was suspended with 125 mg load in a miniature organ bath containing 2 ml of Tyrode's solution and was oxygenated with the 5% CO_2 mixture at 37 °C. After 1 h of equilibration, each tissue was electrically (field) stimulated at 0.1 Hz for 0.4 msec duration and with a supramaximal voltage. Muscle contractions were transduced and recorded with a Grass polygraph. Muscle strips were stimulated for 30 min in INDO at 15–45 μg/ml. Then stimulation was stopped, the bath solution was changed, and was replaced with fresh Tyrode's solution containing an identical amount of INDO and 2 μg/ml of physostigmine. At this time, these tissues were left without stimulation, and the bath solution was collected after 30 min for bioassay. Under similar bath conditions, the tissues were field stimulated at 0.3 Hz, with duration and voltage as before, for 30 min and the bath fluid was again collected for bioassay. Each tissue was blotted dry and weighed. Controls were treated in the same manner but without INDO.

The ACh bioassay method[6] was modified to increase sensitivity 4-fold. The modification consisted of placing the tissue in a 2 ml organ bath and exposing the tissue to 5 μg/ml of morphine and 20 ng/ml of physostigmine. Bioassays were carried out with room lights off.

Results and discussion. The control longitudinal muscle strip with attached Auerbach's plexus ($n = 18$) maintained twitch tension for 30 min without detectable tension loss during electrical stimulation. The tissue in INDO, however, failed to maintain their twitch tension. At 15, 30, and 45 μg/ml INDO, the twitch tension was depressed by 38%, 40% and 64%, respectively ($n = 6$ per dose), compared with the corresponding pre-drug twitch tension. That INDO blocks supramaximally stimulated twitch tension substantiates the finding of EHRENPREIS et al.[5], even though they only used a submaximal stimulus strength.

Blockade of the twitch tension usually occurred within 5 to 7 min although the tissues remained in contact with the INDO for 30 min. The effects of INDO (15–45 μg/ml) on spontaneous transmitter output from Auerbach's plexus was determined in the presence of INDO and the results are shown in Table I. The ACh output from INDO treated tissues is not different from that of the controls. The independent t-test values, with increasing drug concentrations, are 0.0025, 0.1525, and 0.6447 compared with respective controls; t-values are not statistically significant ($p > 0.05$). These results offer direct proof that PGE has no physiological role in the liberation of ACh from ACh-containing neurons of Auerbach's plexus of guinea-pig ileum. Because if PGE does have any physiological role in

[1] J. HAZRA, Eur. J. Pharmac. *11*, 395 (1970).
[2] H. H. JASPER and J. TESSIER, Science *172*, 601 (1971).
[3] E. F. DOMINO and M. STAWISKI, Res. Commun. Chem. Path. Pharmac. *2*, 461 (1971).
[4] Å. WENNMALM and P. HEDQVIST, Life Sci. *10*, 465 (1971).
[5] S. EHRENPREIS, J. GREENBERG and S. BELMAN, Nature New Biol. *245*, 280 (1973).
[6] W. D. M. PATON and E. S. VIZI, Br. J. Pharmac. *35*, 10 (1969).

Table II. Effect of indomethacin on the field stimulated acetylcholine output from Auerbach's plexus of guinea-pig ileum

	Indomethacin concentration (μg/ml) 0	15	30	45
Animal group[b]	Acetylcholine output: (ng/g/min)[a]			
I(6)[c]	51.57 ± 10.61	36.33 ± 5.03		
II(6)	45.20 ± 4.84		36.52 ± 6.68	
III(6)	57.77 ± 14.77			38.10 ± 6.04

[a] Same as in Table I. [b] Same as in Table I, with the following exception: After the 2nd 30 min incubation period the bath fluid was replaced again with fresh fluid containing physostigmine ± indomethacin and a 3rd 30 min incubation was performed. During the 3rd incubation period the tissue was field stimulated supramaximally at 0.3 Hz with 0.4 msec duration. At the end of this 3rd incubation, this bath fluid was assayed for acetylcholine content. [c] Same as in Table I.

ACh liberation, then the effect of PGE synthesis blockade should be visible in the spontaneous ACh output from Auerbach's plexus. This output should be affected because Auerbach's plexus possesses complete neuron, that is, neuron with cell body and processes. And, within the realm of neuron and chemical transmission theory, this would mean that spontaneous ACh output is the result of the activity of the ACh-containing neurons of the Auerbach's plexus. If, therefore, PGE does have any physiological role in ACh liberation, PGE synthesis inhibition should have affected significantly the spontaneous ACh output from ACh-containing neurons. Furthermore, after the inception of these studies, a report by BOTTING and SALZMANN[7] showed that INDO at 10 μg/ml reduced PGE output from whole ileum of guinea-pig to undetectable levels but that INDO in concentrations as high as 20 μg/ml was ineffective in changing ACh output in the majority of cases. Incidentally, these workers did not use proper control in their ACh output measurement.

Thus the findings presented here make it most unlikely that PGE is involved in ACh liberation from Auerbach's plexus of guinea-pig ileum, refute the hypothesis of EHRENPREIS et al.[5], and negate the hypothesis of WENNMALM and HEDQVIST[4] that PGE by a negative feed-back controls cholinergic transmission. If the latter was the case, then after the blockade of PGE synthesis by INDO, the ACh output should have increased. As can be seen (Table I), there is no increase in ACh output after INDO.

Since EHRENPREIS et al.[5] concluded that PGE has a role in ACh liberation on the basis of the effects of INDO on end-organ response (i.e., muscle contractions) induced by field stimulation, the effects of INDO on ACh output caused by field stimulation was also extended. INDO at concentrations of 15, 30, and 45 μg/ml failed to block significantly the ACh output resulting from field stimulation as shown in Table II. The independent *t*-test values, with increasing drug concentrations, are 1.2984, 1.0518, and 1.2343 compared with respective controls and are not statistically significant ($p > 0.05$). The fact that INDO, at a concentration of 45 μg/ml, caused a statistically significant block in twitch tension induced by field stimulation (present study as well as a previous study[5]), and yet failed to alter ACh output caused by field stimulation, clearly indicates how EHRENPREIS et al.[5] could have reached to a wrong conclusion based on end-organ effect of INDO, a PGE synthesis inhibitor.

Summary. The effect of INDO, a PGE synthesis inhibitor, on ACh output from Auerbach's plexus of guinea-pig ileum was investigated. INDO (15–45 μm/ml) failed to alter significantly either spontaneous ACh output or ACh output induced by field stimulation. It is concluded that PGE plays no physiological role in ACh liberation from this tissue.

J. HAZRA[8]

Neurophysiology and Behavioral Research Branch, National Environmental Research Center, EPA, Research Triangle Park (North Carolina 27711, USA), 30 December 1974.

[7] J. H. BOTTING and R. SALZMANN, Br. J. Pharmac. *50*, 119 (1974).

[8] I thank J. GREENBERG and J. E. COMATY for their skillful assistance in the preparation of the longitudinal muscle strip and drug solutions. The research was done at New York State Res. Inst. for Neurochem. and Drug Addict., Ward's Isl., New York, and was supported in part with personal fund.

Hepatic Levels of Cyclic AMP in Normal and Lead-Sensitized Rats after Treatment with Bacterial Endotoxin

Some recent reports suggest that bacterial endotoxin may alter cyclic adenosine-3′,5′-monophosphate (cAMP) metabolism, thus explaining the variety of cellular and metabolic events which occur during endotoxin shock. For example, it has been shown that injection of endotoxin into rats and baboons causes rapid glycogenolysis in the liver, followed by increased blood glucose levels, and finally, hypoglycemia[1,2]. These observations could readily be explained if circulating endotoxin increased hepatic cAMP levels, thereby causing phosphorylase activation and glycogen degradation. Results reported by BITENSKY et al.[3] provide direct evidence that *E. coli* endotoxin interacts with hepatic cell membranes to increase the responsiveness of adenyl cyclase to epinephrine, and GIMPEL[4] found increases in hepatic adenyl cyclase activity in guinea-pigs treated with endotoxin. For these reasons, experiments were designed to measure hepatic cAMP levels in rats after i.v. injections of endotoxin. Cyclic nucleotide levels were also determined, using lead-sensitized animals, since lead acetate[5,6] is known to markedly sensitize rats to endotoxin.

[1] J. P. FILKINS, Proc. Soc. exp. Biol. Med. *142*, 915 (1973).
[2] K. HOLPER, R. A. TREJO, L. BRETTSCHNEIDER and N. R. DI LUZIO, Surg. Gynec. Obstet. *136*, 593 (1973).
[3] M. W. BITENSKY, R. E. GORMAN and L. THOMAS, Proc. Soc. exp. Biol. *138*, 773 (1971).
[4] L. P. GIMPEL, D. S. HODGINS and E. D. JACOBSON, Clin. Res. *19*, 475 (1971).
[5] R. B. JONES, J. L. WISE and L. A. KIESOW, Infect. Immun. *8*, 683 (1974).
[6] H. SELYE, B. TUCHWEBER and L. BERTÓK, J. Bact. *91*, 884 (1966).