In vitro Demonstration of Seasonal Transition Effect on Acetylcholine Liberation from Auerbach's Plexus of Non-Hibernator

During an investigation of the possible role of prostaglandin E in the liberation of acetylcholine (ACh) from ACh neurons of the Auerbach's plexus of guinea-pig ileum[1], I accidentally discovered that ACh neurons of the Auerbach's plexus of guinea-pig ileum have an innate sensing mechanism through which they respond uniquely to the approach of the summer season causing an abrupt rise of the ACh output towards the end of spring. The sudden rise of the ACh output began 3 weeks before the beginning of summer, reached a peak 2 weeks prior to the initiation of summer, and then declined during the remaining 2 weeks. The results of a study of this serendipitous finding are presented in this article.

Methods. Charles River guinea-pigs of both sexes 275–450 g) were obtained from the supplier at least 1 week before the experiment began and were housed in a temperature controlled room (73 ± 5 °F). The small intestine was dissected according to the method described previously[2]. Although 2 adjacent segments of ileum from each animal were used to prepare 2 strips of longitudinal muscle with attached Auerbach's plexus (one acting as control, the other for drug treatment[1]) it should be considered that each animal only provided 1 test strip, because only the results for controls in some previously published[1] and some unpublished data will be presented here.

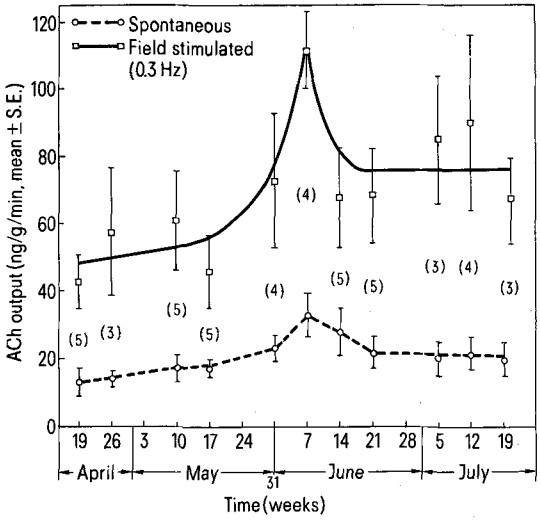

Fig. 1. Acetylcholine ouput of Auerbach's plexus of guinea-pig ileum at 37 °C between April 12 and July 19, 1974. Study period divided into weeks with last day of week designated as the time. Number within parenthesis is number of animals. 2 or less experiments per week were not included.

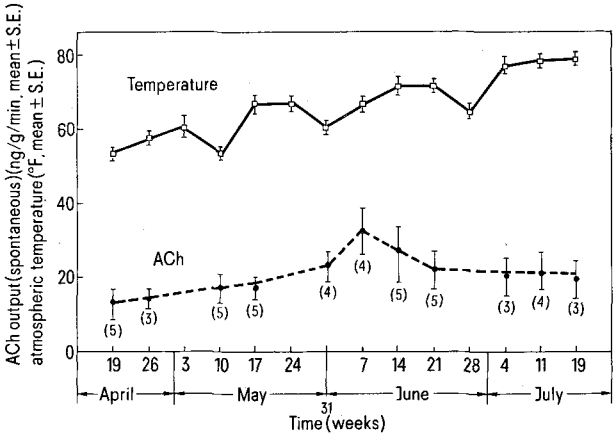

Fig. 2. Spontaneous acetylcholine output of Auerbach's plexus of guinea-pig ileum at 37 °C and its relation to corresponding atmospheric temperature (local atmospheric temperature data obtained from the United States Climatic Center). Number within parentesis is number of animals. Time designation is same as in for Figure 1.

Each longitudinal muscle strip with attached Auerbach's plexus was set up in a small organ bath containing 2 ml of Tyrode's solution and oxygenated with 5% CO_2 mixture at 37 °C. The tissue was stretched with 125 mg load and allowed to equilibrate for about 1 h, after which the experiment was begun. For determination of the spontaneous ACh output, the tissue was incubated in Tyrode's solution containing 2 μg/ml of physostigmine sulphate. After 30 min of incubation, the bath fluid was collected and stored in crushed ice for analysis of the ACh content by bioassay, which was performed on the same day. Then, the tissue with the organ bath was rinsed twice with fresh Tyrode's solution and reincubated in Tyrode's solution containing 2 μg/ml of physostigmine sulphate. This time, the tissue was field-stimulated at 0.3 Hz with 0.4 msec pulse of supramaximal voltage (which was determined at the outset for each tissue based on the muscle tension amplitude, a pulse rate of 0.3 Hz was used because preliminary experiment showed no difference of the ACh output between the spontaneous and the 0.1 Hz field-stimulation). After 30 min of incubation and stimulation, the bath fluid was collected again and stored. The tissue was blotted dry and weighed.

ACh analysis was done on an isolated guinea-pig ileum using the bioassay method described previously[1]. Unknown samples were bracketed between known concentrations of ACh bromide. ACh bromide was dissolved in Tyrode's solution containing 2 μg/ml of physostigmine sulphate.

Results and discussion. ACh output from Auerbach's plexus with attached longitudinal muscle of guinea-pig ileum was determined between April 12 and July 19, 1974. The mean spontaneous ACh output increased every week until the week that ended on May 31 (Figure 1) as compared with that of the initial period (i.e., the week that ended on April 19), but the increase was not statistically significant ($p > 0.05$). Between May 31 and June 21, however, the mean spontaneous ACh output increased sharply reaching a peak by the week ending on June 7 and then dropped abruptly reaching a mean ACh output identical to that of May 31 by June 21 (Figure 1). Even though the spontaneous ACh output values for both June 7 and 14 were higher than that of April 19, only the value for June 7 was significantly different from the latter ($p < 0.05$). During the remainder of the period (weeks ending between June 21 and July 19; Figure 1), the spontaneous ACh output level did not vary; however, the mean ACh output level was higher than that of April 19, but the difference was not significant ($p > 0.05$).

Although the spontaneous ACh output for June 7 was significantly different ($p < 0.05$) from that of April 19 (Figure 1), this seemed, at first, to be merely coincidental. However, the ACh output in response to field-

[1] J. HAZRA, Experientia *31*, 565 (1975).
[2] W. D. M. PATON and E. S. VIZI, Br. J. Pharmac. *35*, 10 (1969).

stimulation, determined from the same tissue samples, makes a mere coincidence most unlikely. Because the field-stimulated ACh output showed also a similar increase during that time period (Figure 1). In fact, the value of the field-stimulated ACh output that differed significantly ($p < 0.05$) from that of April 19 was found only in the week ending on June 7. Thus, an increase of the spontaneous ACh output in other words, an increase of ACh neuronal activity of the Auerbach's plexus during the week ending on June 7 in comparison with that of April 19 (Figure 1) is real. Because the week ending June 7 was 2 weeks prior to the beginning of summer 1974 and because the ACh output was not related to known geophysical effect, e.g., temperature (Figure 2),

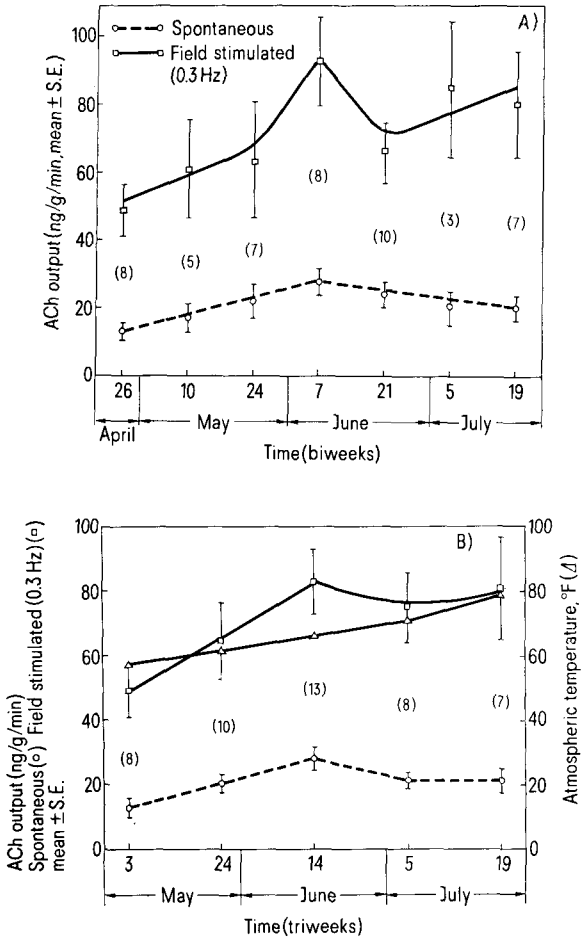

Fig. 3. Weekly data of Figure 1 and those that were excluded from Figure 1 composited and plotted on the basis of biweeks (A) and triweeks (B). Mean atmospheric temperature of each triweek period is also shown in (B). Number within parenthesis is number of animals.

it is likely that these neurons on one hand retained some common genetic component in spite of evolutionary changes, on the other respond to the approach of summer with a transient outburst of activity, although the guinea-pig is a non-hibernator[3].

This finding is unique; there is no published report on any neurotransmitter output change of this pattern either in a hibernator or a non-hibernator. There is, however, a published report on seasonal variation of the ACh output from brain during sleeping and waking in a non-hibernator. MONNIER and HERKERT[4] observed that the ACh output from the rabbit brain varied according to the season and that there was a good correlation between the ACh output and the external daily temperature, a parameter of seasonal condition used by these researchers. Because the over-all spontaneous ACh output from the Auerbach's plexus of guinea-pig ileum showed no relation to the external daily temperature (Figure 2), and because the spontaneous ACh output showed no change during the summer (Figures 1 and 2; summer began June 21 according to the United States Climatic Center), it is apparent that ACh neurons of the Auerbach's plexus from these guinea-pigs responded only to the seasonal transition from the spring to the summer. To demonstrate this transitional reaction, the weekly output data was composed and plotted in Figure 3 on a biweekly (3A) and a triweekly (3B) basis. As can be seen, there is a linear rise of the spontaneous ACh output up to 2 weeks (3A) or 1 week (3B) before the beginning of summer. Again, both points are significantly different ($p < 0.05$) from that of the corresponding initial point. Moreover, such a parallelism between the spontaneous and the field-stimulated curves of ACh output (3B) is difficult to reconcile as an epiphenomenon. Thus, data presented, suggest the existence of a seasonal transition effect on ACh output. Whether this seasonal transition response is common to all breeds of guinea-pigs and whether it varies from year to year are questions yet to be resolved.

Summary. Acetylcholine release from Auerbach's plexus of guinea-pig ileum, in vitro, both spontaneous and evoked by field-stimulation, responds to the seasonal transition from the spring to close to the beginning of summer. It did not change during the summer, however, tended to be higher than during the initial period (3 weeks after the beginning of spring).

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An Evaluation of Apomorphine Action on Dopaminergic Receptors

A large number of behavioural, biochemical and electrophysiological studies provide considerable evidence to prove that apomorphine has a direct action on central dopaminergic receptors. Thus, apomorphine induced decreased dopamine turnover in corpus striatum[1], modified stereotype behaviour in 6-OHDA-treated rats[2], pecking response in pigeons[3] and gnawing behavior in rats[4] are reported to be a direct effect of the drug on the dopaminergic neurones of CNS.

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