Effects of Cerebral Stimulation on the Biosynthesis *in vitro* of Nucleotides and RNA in Brain

In view of the possibility that memory, such as that acquired in learning, is represented by some permanent change in the brain, and that the change is brought about as a result of chemical activity in the brain cell, we carried out experiments to observe whether various stimulating agents affect nucleotide and RNA biosynthesis in isolated brain. We examined the effects of electrical stimulation, and of changes in the cation composition of the incubation medium and of acetylcholine, on the rates of incorporation of labelled uridine into uridine nucleotides and RNA in slices of rat brain cortex. The results are summarized here. Full details will be published elsewhere.

Slices of cerebral cortex (0·3–0·4 mm thick) from adult female rats were incubated at 37° C in Krebs–Ringer phosphate media containing 10 mM glucose in the presence of oxygen by the conventional Warburg manometric technique. Uridine-2-^{14}C (usually about 6 μM) was added to the media after temperature equilibration; after incubation for 30 min the slices were washed and homogenized at 0° C in 5 per cent trichloracetic acid. The homogenate was centrifuged and the supernatant analysed chromatographically for phosphorylated and free uridine after removal of trichloracetic acid by ether. After removal of acid soluble radioactivity by washing and lipid removal by alcohol–ether extraction, the residue from the centrifuged homogenate was dissolved in 2 ml. of 0·2 N KOH and incubated at 37° C for 18 h. DNA and proteins were precipitated with perchloric acid and the radioactivity of the hydrolysed RNA in the supernatant was estimated in a liquid scintillation counter. Electrical stimulation of the slices of brain cortex was carried out by methods already described[1].

Application of electrical impulses had a marked diminishing effect on the rate of conversion of uridine into uridine polyphosphates and into RNA in slices of rat brain cortex[2,3]. The potent neurotoxin, tetrodotoxin (5 μM), blocked the inhibitory effect of electrical impulses on cerebral RNA biosynthesis. Because the metabolic effects of electrical impulses in isolated brain were suppressed by the addition of small concentrations of tetrodotoxin[1]—which acts by inhibiting sodium influx during electrical stimulation—it seemed possible that the suppressing action of electrical impulses on the formation of uridine polyphosphates and RNA was caused by the influx of sodium ions into the brain cell. This prediction was verified by our finding that increasing the concentration of sodium ions in the bathing medium above an initial value of 128 mequiv/l. led to a diminution of the rate of incorporation of labelled uridine into both RNA and uridine polyphosphates. For example, an increase of the NaCl content of the medium from 128 mM to 178 mM diminished the rate of incorporation of uridine into uridine polyphosphates by 47 per cent and into RNA by 56 per cent. The influx of uridine into the brain tissue, however, was not diminished. Ouabain (1 μM), which suppresses the activity of the sodium pump, inhibited the incorporation of uridine into RNA by 32 per cent.

Increasing the concentration of KCl in the bathing medium from 5 mM to 15 mM led to an increase in the rate of incorporation of uridine into RNA by 54 per cent; further increase to 50 mM brought about a considerable fall in the rate of incorporation of uridine into RNA. Two effects of potassium ions on RNA biosynthesis seemed to operate: (1) a stimulation dependent on the cell content of K^+; (2) an inhibition caused by the fall in cell level of ATP that occurs with relatively high concentrations of K^+ in the medium[4].

Studies with dialysed brain extracts in the presence of added magnesium ions and ATP showed that concentrations of sodium chloride of 100 mM or over led to diminished rates of incorporation of uridine into uridine polyphosphate (though not into uridine monophosphate), the diminution amounting to over 39 per cent of the rate found in the absence of sodium ions. Potassium ions acted similarly to sodium ions in the brain extracts. The stimulating action of potassium chloride at 15 mM, on incorporation of uridine into RNA in brain slices, was not the result of an accelerating action of potassium ions on the formation of uridine polyphosphates but rather of an increased retention of potassium ions in the brain cell leading to an increased rate of formation of ATP (by their well known accelerating effect on the activity of pyruvate phosphokinase) or perhaps to a direct effect on RNA biosynthesis[5].

In other experiments, acetylcholine (1 mM), in the presence of eserine, brought about an increased rate of incorporation of uridine into RNA in rat brain slices in the presence of potassium ions. The combination of 15 mM KCl and acetylcholine brought about a doubling of the rate of uridine incorporation into RNA found in the presence of a normal Ringer medium. Carbamyl-choline (in the absence of eserine) was as effective as acetylcholine (in the presence of eserine). The stimulant action of acetylcholine was diminished in the absence of sodium ions. Results of experiments on the kinetics of uridine incorporation indicated that the effect of acetylcholine may be a result of its diminution or removal of the initial time lag[6] observed during the course of uridine incorporation into RNA. Acetylcholine did not affect the diminished rate of RNA biosynthesis brought about by electrical impulses.

It seems therefore that the cerebral stimulating agents we have described promote changed rates of nucleotide and RNA biosynthesis in rat brain slices. The effects of these stimulating agents seem to stem largely from reactions at the brain cell membrane.

We thank the Medical Research Council of Canada for financial support.

Carol Prives*
J. H. Quastel

Neurochemistry Section,
Kinsmen Laboratories,
University of British Columbia,
Vancouver 8.

Received November 5, 1968; revised January 20, 1969.

* Present address: Department of Molecular Biology, Albert Einstein College of Medicine, 1300 Morris Park Avenue, Bronx, New York 10461.

[1] Chan, S. L., and Quastel, J. H., *Science*, **156**, 1752 (1967).
[2] Prives, C., and Quastel, J. H., *Proc. Canad. Fed. Biol. Soc.*, **10**, 31 (1967).
[3] Orrego, F., *J. Neurochem.*, **14**, 851 (1967).
[4] Abadom, P. N., and Scholefield, P. G., *Canad. J. Biochem. Physiol.*, **40**, 1603 (1962).
[5] Barondes, S. H., *J. Neurochem.*, **11**, 663 (1964).
[6] Guroff, G., Hogans, A. F., and Udenfriend, S., *J. Neurochem.*, **15**, 489 (1968).

Artefact in the Measurement of the Molecular Weight of Pulse Labelled DNA

Tsukada *et al.*[1] have recently reported that, after denaturation, newly replicated DNA from regenerating rat liver cells apparently has a lower molecular weight than the main body of the chromosomal DNA. After pulse labelling with tritiated thymidine, they isolated the DNA by conventional methods, which extensively shear the DNA, thereby reducing its chain length, and then measured the molecular weight of the denatured DNA by sedimentation on an akaline sucrose gradient.

We have obtained exactly the same results with a murine lymphoma cell (L5178Y) grown in tissue culture, and have shown that there is no need to invoke any special intermediate which is involved in DNA replication in order to explain the experimental results. The peak molecular weight of pulse labelled DNA will always be