an early introduction of conservation measures.

Research on methods of improving the efficiency of energy use and on means of implementing these improvements is just beginning, and more is needed. But it seems likely that from now on energy will always cost more and that expensive energy will induce some consumers to do with less and others to use it more efficiently. As a result, energy needs in this country may well be grossly overestimated. Moreover, it seems clear that in the long run energy needs could be reduced still further through effective conservation policies.

—ALLEN L. HAMMOND

References

1. D. Chapman, T. Tyrell, T. Mount, *Science* **178**, 703 (1972).
2. W. E. Mooz and C. C. Mow, *California's Electric Quandary: Estimating Future Demand* (Rand Corporation, Santa Monica, 1972).
3. W. Heller, quoted in *Conservation of Energy* (Committee on Interior and Insular Affairs, U.S. Senate, Washington, 1972).
4. R. D. Doctor *et al.*, *California's Electricity Quandary: Slowing the Growth Rate* (Rand Corporation, Santa Monica, 1972).

Cyclic AMP in Brain: Role in Synaptic Transmission

The task of investigating the function of an organ as complex as brain is enormously difficult, but it promises proportionate intellectual and practical rewards. The ultimate goal—the explanation of human behavior in terms of cellular biochemistry—may not be achieved in the foreseeable future. Nevertheless, as an increasing number of investigators devote their time and talents to the neurosciences, they are beginning to accumulate clues to how the intricate circuitry of the central nervous system transmits and integrates nerve impulses. One area of research that has experienced explosive growth in the last 5 years concerns the role of 3′,5′-adenosine monophosphate (cyclic AMP) in brain. Although the story is by no means complete, the formation and degradation of cyclic AMP have been shown to be regulated, at least partially, by the same factors that affect impulse conduction by neurons. In addition, some central neurons appear to transmit their messages to other nerve cells by releasing chemicals, called neurohormones, that diffuse across the synapses and stimulate the production of cyclic AMP by the target cells.

Since Earl W. Sutherland, who is now at Vanderbilt University in Nashville, Tennessee, discovered cyclic AMP and elucidated its role as the "second messenger" for the hormones epinephrine and glucagon, this ubiquitous chemical has been shown to play a central role in numerous cellular and hormonal activities (*1*). Theodore Rall, at Case Western Reserve University in Cleveland, Ohio, collaborated with Sutherland on the earlier research. According to Rall and Sutherland, brain is an unusually rich source of adenylate cyclase, the enzyme that catalyzes the synthesis of cyclic AMP from adenosine triphosphate (ATP), and also of phosphodiesterase, the enzyme that inactivates the cyclic nucleotide.

Rall and his colleagues are currently investigating the factors that regulate cyclic AMP synthesis and degradation in brain slices. They have found that stimuli known to cause depolarization of neurons produce increases in the cyclic AMP concentrations of the slices. As a result of depolarization, neurons either fire or become more responsive to subsequent stimulation. The stimuli they are studying include putative neurohormones—like norepinephrine, serotonin, and histamine—and electrical stimulation. Rall thinks that the neurohormones bind to receptors on the cell membrane and thus stimulate the activity of adenylate cyclase. Electrical stimulation, on the other hand, probably acts indirectly by causing the release of adenosine.

Role of Adenosine

Adenosine, which is not thought to be a neurohormone, produces striking increases in the cyclic AMP concentrations of brain slices. According to Rall, adenosine stimulates adenylate cyclase by binding to a specific membrane receptor that is not affected by the neurohormones. He can distinguish the adenosine receptor from the others by use of the chemical theophylline. In most systems, theophylline potentiates the observed increases in cyclic AMP concentrations because it inhibits the activity of phosphodiesterase; however, it prevents the increases elicited by both electrical stimulation and adenosine, presumably by blocking the adenosine receptor.

John W. Daly and his colleagues at the National Institute of Arthritis, Metabolism, and Digestive Diseases in Bethesda, Maryland, are also interested in the factors that control cyclic AMP formation and degradation in brain slices. They have observed that complex chemical depolarizing agents like ouabain, veratridine, and batrachotoxin enhance the conversion of ATP to cyclic AMP. These chemicals also cause the release of adenosine from the slices. Because the adenosine release parallels the enhanced formation of cyclic AMP and because theophylline antagonizes the effects of the depolarizing agents, Daly has postulated that these agents also act indirectly through adenosine.

According to Daly, adenosine and adenosine-dependent depolarizing agents interact synergistically with the neurohormones, histamine, serotonin, and norepinephrine, to produce a much greater enhancement of cyclic AMP formation than would be caused by either kind of stimulus acting alone. He has speculated that this type of synergism may help to regulate or modulate the activity of neurons in the brain. Informational input to the same neuron from two or more sources could cause a much greater cyclic AMP response than that caused by a single source. Evaluation of this hypothesis requires a better understanding of the subsequent biological functions of cyclic AMP in the brain.

Although brain slice techniques are valuable for studying the regulation of cyclic AMP metabolism, they entail two major problems for the investigator. The slices, even of a restricted area of the brain like the brainstem or cerebellum, are composed of several cell types. These include the neurons that actually transmit impulses, glial cells, whose functions are not well understood at all, and the cells of connective tissue. The investigators have not yet been able to determine which cell types are responsible for the observed changes in cyclic AMP concentrations in brain slices, and thus they have little information about cyclic AMP's function there. The second problem is that there is no way to correlate these biochemical changes with the behavior, including learning and memory processes, of the living animal.

In order to solve the first problem, several laboratories have directed their efforts to the study of brain cells in culture. For example, Alfred G. Gilman, first with Marshall Nirenberg at

the National Heart and Lung Institute in Bethesda, Maryland, and now at the University of Virginia School of Medicine in Charlottesville, has been investigating the effects of neurohormones and other substances on cultured neuroblastoma and glial cells. A neuroblastoma is a tumor of the nervous system that consists mainly of embryonic nerve cells; some lines, however, do possess many of the electrical and biochemical properties of differentiated neurons. According to Gilman and Nirenberg, none of the four neuroblastoma strains treated with isoproterenol (which is chemically and biologically similar to norepinephrine), histamine, or adenosine had increased amounts of cyclic AMP. Only prostaglandins of the E series, especially prostaglandin E_1, stimulated the synthesis of the nucleotide. These investigators found, however, that norepinephrine treatment produced striking increases in the cyclic AMP concentrations of glial tumor cells. Gilman also found that norepinephrine increased the cyclic AMP concentrations of mixed cultures of normal fetal brain cells. The increases appeared to be directly related to the proportion of glial cells in the preparations. Therefore, Gilman has concluded that the cyclic AMP response may be attributed to the glia rather than to the neurons.

Several scientists contend that neuroblastoma cells are capable of dividing and may not be representative of normal mature neurons that cannot divide. Moreover, neuroblastoma cells respond physiologically not to norepinephrine but to acetylcholine, a neurohormone that does not increase cyclic AMP concentrations; they may not have receptors for, or the capability to react to, norepinephrine. According to Rall and to Daly, there are also significant differences between the responses of brain slices and cultured glial tumor cells to norepinephrine and adenosine. Thus, it is still somewhat difficult to extrapolate from the events occurring in these relatively simple systems to those taking place in intact brain.

Floyd E. Bloom and his colleagues George Siggins and Barry Hoffer, at the National Institute of Mental Health research laboratory at St. Elizabeths Hospital in Washington, D.C., have circumvented the extrapolation problem by using intact brain to perform their investigations into the function of cyclic AMP in the cerebellum. They have concentrated their research on the regulation of the firing of the Purkinje cells of the cerebellar cortex. The axons of these neurons are

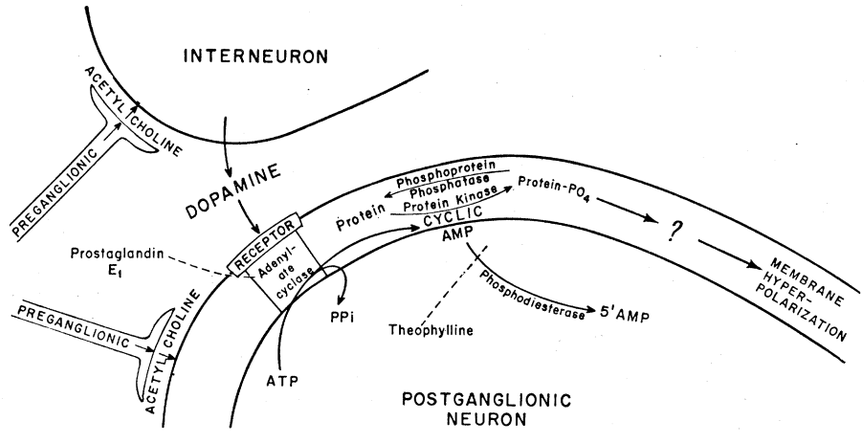

Fig. 1. Scheme of a mechanism of action proposed for cyclic AMP in the dopamine-mediated transmission of impulses across synapses in the cervical ganglion of the sympathetic nervous system. [Source: P. Greengard, Yale University School of Medicine]

the only ones to leave the cerebellar cortex to communicate with other parts of the brain. Bloom found that application of norepinephrine to the Purkinje cells with a micropipette electrode altered the characteristic firing pattern of the neurons in a specific manner—long pauses interrupted the rapid discharge pattern of the cells. Using an intracellular microelectrode, he determined that the cells had become hyperpolarized. As a result of hyperpolarization—which is the opposite of depolarization—neurons become less responsive to stimulation. During the period of hyperpolarization, the inside of the cell has more negative charges than usual. This is ordinarily caused by a decreased resistance of the cell membrane to the outward flow of potassium ions or to the inward flow of chloride ions; in this case, however, resistance to the flow of the ions increased. Although Bloom does not yet understand how norepinephrine produces this effect, it does enable him to distinguish the action of norepinephrine on Purkinje cells from that of other inhibitory neurohormones.

Norepinephrine-Containing Cells

After this demonstration of the response of Purkinje cells to norepinephrine, Bloom used a histochemical technique to identify norepinephrine-containing neurons that originate in a part of the brain called the locus coeruleus and synapse with the Purkinje cells. Stimulation of the cells of the locus coeruleus altered the firing pattern of the Purkinje cells in exactly the same way as did direct application of norepinephrine. If the synthesis of norepinephrine was inhibited or if the norepinephrine-containing neurons were selectively destroyed by injection of 6-hydroxydopamine, stimulation of the locus coeruleus had no effect on the firing pattern of the Purkinje cells.

According to Bloom, the effects of norepinephrine are mediated by cyclic AMP. Cyclic AMP, applied to the Purkinje cells by micropipette, mimicked the effects of norepinephrine on the firing pattern. The presence of inhibitors of phosphodiesterase potentiated the effects of both norepinephrine and cyclic AMP. Prostaglandins E_1 and E_2, which are known to reduce cyclic AMP concentrations in some cells, prevented the action of norepinephrine in rat cerebellum. Finally, Bloom used an immunofluorescent histochemical technique, which is specific for cyclic AMP, to show that the cyclic AMP content of Purkinje cells did indeed increase both when norepinephrine was applied directly to the cells or when the locus coeruleus was stimulated.

Bloom's current research is expanding in two directions. For one, he is attempting to correlate behavior with changes in the firing patterns of the neurons. He has found that the norepinephrine neurons of the locus coeruleus of unrestrained, unanesthetized cats fired slowly during drowsy periods and in the early stages of sleep. During rapid eye movement sleep, which is the phase of sleep associated with dreaming, the nerve discharge displays a pattern of rapid bursts of activity. In attentive wakefulness, the pattern is a fairly regular but rapid series of discharges. Bloom states that these alterations in the firing patterns of the locus coeruleus do not necessarily produce the behavioral changes, but this brain center may be activated when it is important for the animal to be responsive to its environment and

may be suppressed when sleep occurs.

Bloom would also like to determine the molecular effects of increased cyclic AMP concentrations in neurons. In order to achieve this objective, he is trying to culture mature Purkinje cells and to study the effects of cyclic AMP on certain enzyme systems and on gene expression by these cells.

Elucidation of the many roles of cyclic AMP has stimulated the search for a fundamental biochemical mechanism that can explain its diverse effects. Paul Greengard and his colleagues at the Yale University School of Medicine in New Haven, Connecticut, have proposed one such unifying mechanism. They identified a member of a family of enzymes called the protein kinases in mammalian brain. These enzymes catalyze the transfer of the terminal phosphate group of ATP to an acceptor protein. Cyclic AMP causes a marked stimulation of the activity of protein kinases, which are widely distributed throughout the animal kingdom. Greengard has hypothesized that cyclic AMP may produce its many different effects because of its ability to stimulate various protein kinases, with resultant phosphorylation of specific proteins. The natural protein substrates for the kinases are for the most part unknown; however, histones, basic proteins associated with chromosomal DNA and thought to be involved in the regulation of gene expression, are good substrates for the cyclic AMP–dependent protein kinases. Moreover, Greengard has recently reported that a minor protein component of synaptic membrane fractions from rat cerebrum is specifically phosphorylated in the presence of cyclic AMP. The synaptic fragments also contain protein kinase and a phosphoprotein phosphatase, an enzyme that is able to remove phosphate groups from proteins.

In his investigations of the possible role of cyclic AMP in the transmission of impulses across synapses, Greengard initially studied a relatively simple preparation of peripheral nervous tissue and later studied the caudate nucleus of brain. The peripheral preparation, a ganglion of the sympathetic division of the autonomic nervous system, contains three types of nerve cells (Fig. 1): the preganglionic (input) neurons that originate in the spinal cord and release acetylcholine as their chemical transmitter to neurons within the ganglion; interneurons that are innervated by the input neurons and that release dopamine; and postganglionic (output) neurons that receive synapses directly from the input neurons as well as from the interneurons. The output neurons ultimately innervate target organs outside the ganglion. He observed that stimulation of the input neurons produced an increase in the cyclic AMP content of the ganglion. Other experiments indicated that the increase required the synaptic transmission of nerve impulses and that dopamine, not acetylcholine, was directly involved in producing this effect. In fact, direct application of dopamine to the ganglionic tissue also resulted in higher concentrations of cyclic AMP.

Dopamine Receptors

Greengard proposed that the dopamine released by the interneuron during the period of stimulation combines with specific receptors on the postsynaptic membrane, stimulates adenylate cyclase, and thus increases the concentration of cyclic AMP in the postsynaptic cell. Accumulation of cyclic AMP results in hyperpolarization of the postganglionic neuron—an effect also produced by direct application of dopamine or cyclic AMP. Thus, the cell becomes less responsive to subsequent direct excitatory stimulation by the preganglionic neuron. Greengard hypothesizes that this provides a mechanism for the inhibitory modulation of the synaptic transmission of nerve impulses through the sympathetic ganglion.

The molecular effects of the cyclic AMP that may be generated as a result of synaptic transmission are not well understood. Greengard, however, has speculated that stimulation of protein kinases by cyclic AMP may result in both transient and persistent alterations—including the establishment of short- and long-term memory—in the properties of neurons. Phosphorylation of a membrane protein, such as the one found in the synaptic preparations of rat cerebrum, might result in changes in membrane properties and thus produce the observed changes in membrane potential and, possibly, generate short-term memory. The changes in membrane potential could then be reversed by the phosphoprotein phosphatase removing the phosphate from the protein. Histone phosphorylation could be involved in long-term effects, including establishment of long-term memory, if such phosphorylation does indeed cause a change in gene expression and therefore in protein synthesis in nerve cells.

Greengard and his colleagues have recently extended their investigations from the peripheral nerve preparation to the caudate nucleus of rat brain. There, they have found an adenylate cyclase with properties similar to the one they previously studied in ganglionic tissue. Low concentrations of dopamine, which occurs naturally in the caudate nucleus, stimulated the activity of the adenylate cyclase; chlorpromazine and haloperidol, potent antipsychotic agents widely used in the medical treatment of schizophrenia and other psychoses, antagonized the effects of dopamine.

The caudate nucleus is involved in the coordination of muscular activity. In the human, Parkinson's disease is associated with a depletion of dopamine within this brain center. According to Greengard, current techniques of searching for new therapeutic agents for the treatment of parkinsonism are not satisfactory. Thus, the adenylate cyclase preparation from the caudate nucleus should be of practical value in finding new drugs that mimic the biochemical action of dopamine; such drugs may be effective in the treatment of parkinsonism. In addition, chlorpromazine and haloperidol produce tremor and other symptoms similar to those of Parkinson's disease because they antagonize the actions of dopamine in the caudate nucleus. The adenylate cyclase preparation may aid in the development of antipsychotic agents without these undesirable side effects.

Norepinephrine and dopamine are structurally related; moreover, both appear to act through cyclic AMP to produce hyperpolarization of nerve cells —norepinephrine in Purkinje cells and dopamine in the output neurons of the sympathetic cervical ganglion. Investigations in Greengard's laboratory are now under way to determine whether dopamine produces similar effects in the neurons of the caudate nucleus.

Numerous questions remain to be answered concerning the role of cyclic AMP in central synaptic transmission of nerve impulses. Current theories may well require extensive modification. Nevertheless, the present pace of research is such that a better understanding of the function of cyclic AMP in the communication between brain cells can be expected.—JEAN L. MARX

Reference

1. E. W. Sutherland, *Science* **177**, 401 (1972).