Technical Papers

Acetylcholine Synthesis[1]

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Acetylcholine participates in the humoral transmission of the effects of nervous stimulation in cholinergic systems. Acetylcholine is released during stimulation of the vagus, the motor nerves to skeletal muscle, the preganglionic fibers of the sympathetic nervous system, and perhaps when impulses cross the synapses of the spinal cord (*1, 2, 4, 5, 10, 13*). It is inferred that acetylcholine is synthesized in the central nervous system and probably also in peripheral nerve tissue, since the amount of acetylcholine liberated during prolonged stimulation may be several times the amount contained in nonstimulated nerve tissue (*9*). Nerve tissues synthesize acetylcholine even *in vitro,* and there is evidence that the synthesis of acetylcholine results from the activity of a specific enzyme (*6–8, 11, 12, 14–16, 32*). The amount of acetylcholine synthesized is probably regulated by the dynamic equilibrium of the various metabolites that surround the enzyme-substrate complex during the process of synthesis.

In the presence of blood serum and spinal fluid more acetylcholine is synthesized by nerve tissue *in vitro* than in the absence of serum and spinal fluid (*16, 17*). Some of the potentiator substances diffuse through Cellophane membrane (*16*). Although serum contains both potentiator and inhibitor substances, the potentiator effect prevails. The potentiator effect may be reduced by various physiological procedures, e.g. less acetylcholine is synthesized in the presence of serum collected from the fatigued arm than in the presence of serum collected from the resting arm (*25*). The effect of the serum and spinal fluid in increasing acetylcholine synthesis is reduced in patients with myasthenia gravis (*16, 17*).

To ascertain the nature of substances having the ability to modify synthesis of acetylcholine the effects of various known metabolites on acetylcholine synthesis *in vitro* were investigated.

Compounds containing energy-rich phosphate bonds (*14, 30*), some products of carbohydrate metabolism (*21*), lower fatty acids (*28*), polypeptides and amino acids (*26*) increase the amount of acetylcholine synthesized. Some decomposition products of nucleic acid (*23*) and of organic phosphates occurring in the body (inosinic acid *23*; ammonia *15, 26*) decrease acetylcholine synthesis.

Of the inorganic ions potassium (*12, 33*), phosphate (*15*), rubidium (*12*), caesium (*12*), barium (*31*), magnesium (*31*), and manganese (*31*) increase the synthesis, whereas potassium in high concentrations (*12*), calcium (*12, 14*), and ammonia (*15, 26*) decrease it.

Hormones also exert an effect on acetylcholine synthesis. Ether extracts of thymus and pancreas decrease the synthesis (*19*), while similar ether extracts of other tissues tested (lymph gland, thyroid, salivary gland, lung, and subcutaneous fat) do not. Since the size of the thymus is regulated by the adrenotrophic hormone of the pituitary gland, this hormone was administered to living animals, and the ability of brain to synthesize acetylcholine was ascertained (*20*). Brain from animals injected with the adrenotrophic hormone synthesized more acetylcholine than brain of noninjected animals. Most steroid hormones decreased the amount of acetylcholine synthesized, but estrogenic hormones and Δ^5 pregnenolone increased the synthesis (*22*). Thyroxine increased the acetylcholine synthesis (*26*), as did epinephrine (*18*). The effect of epinephrine in increasing the amount of acetylcholine synthesized may explain why epinephrine increases the effect of acetylcholine in the central nervous system and improves transmission from nerve to the muscle (*3*).

Vitamins may modify the amount of acetylcholine synthesized. Vitamin E, even in very low concentrations, vitamin C, and most members of the B group increase the amount of acetylcholine synthesized. Vitamin A and K in all the concentrations used, thiamine chloride and vitamin D in higher concentrations decrease the synthesis (*24*).

The variety of the above-enumerated substances suggests that many operate not by a specific action on the enzyme involved in the synthesis of acetylcholine but nonspecifically through other cell processes. Final evaluation of the factors that specifically affect acetylcholine synthesis can be made only after the enzyme system is isolated in pure form.

It is inferred that the enzyme involved in the acetylcholine synthesis contains an active –SH group, since the process is inhibited by agents that decrease the activity of the –SH group (monoiodoacetate, *14, 30*; alloxan, *23*; unsaturated ketones, oxidizing agents, *27*; penicillin, *27*) and is increased by agents protecting the activity of the –SH group (glutathione, cys-

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teine, *26*; antioxidants, *24*; sodium pyrophosphate in low concentrations, *23*). The synthesis can occur aerobically and anaerobically (in the presence of an excess of energy-rich phosphate bonds, e.g. adenosinetriphosphate, *14, 30*). The amount of acetylcholine synthesized depends on temperature (*14, 16*) and pH (optimum at alkaline pH, *29*). The enzyme is located intracellularly (*8*).

On the basis of the above data the following postulate is presented. Normally occurring constituents of cells and extracellular fluid (serum, spinal fluid) modify the amount of acetylcholine synthesized in the living organism. Further, there is a dynamic equilibrium between potentiator substances (organic phosphates, metabolites of carbohydrates and fats, amino acids, inorganic ions, hormones, vitamins) and inhibitor substances (unsaturated and higher fatty acids, aromatic and heterocyclic compounds, steroid substances, inorganic ions, some decomposition products of nucleoproteins and organic phosphates). During physiological activity the original dynamic equilibrium is disturbed, and new dynamic equilibria are established. Certain metabolites of muscle released during prolonged work decrease the synthesis of acetylcholine (*23, 25, 26*). The accumulation of such metabolites is important in the production of fatigue resulting from indirect stimulation and secondary to decreased acetylcholine synthesis.

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Granulocytopenia and Anemia in Rats Fed Diets of Low Casein Content[1]

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Granulocytopenia, correctable by crystalline *L. casei* factor (L.C.F., "folic acid"), has been found to occur occasionally in rats fed highly purified diets (*4*) and regularly when sulfonamides are included in such diets (*3, 4, 7*). Anemia (or impairment in erythropoiesis following hemorrhage) correctable by L.C.F. also has been found in rats fed sulfonamide-containing diets (*1, 3, 6*). Recently we have noted granulocytopenia in rats fed highly purified diets deficient in riboflavin and also among pair-fed, riboflavin-supplemented controls (*5*). Further investigation of this influence of inanition on the production of granulocytopenia has revealed limitation of casein intake to be a highly significant factor.

Weanling albino rats (Osborne and Mendel) were fed one of several purified diets differing only with respect to casein content. Diet No. 1055 contained no casein or protein and consisted principally of Crisco, salt mixture, and dextrose.[2] In the other diets casein (Labco) in varying amounts replaced equivalent weights of dextrose. Total white blood cell counts, polymorphonuclear granulocyte counts, and hematocrit determinations were made as previously described (*6*). For the purposes of this report, granulocytopenia was considered to be present when the polymorphonuclear granulocytes numbered 500 or less per cu. mm. Anemia was considered to be present when the hematocrit was less than 30 vol. per cent.

Of 89 rats fed the casein-free diet (No. 1055), 10

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[2] The casein-free diet No. 1055 consisted of anhydrous dextrose, 86.76 grams; Crisco, 8.0 grams; salt mixture No. 550[3], 4.0 grams; ferric citrate, 1.16 grams; and copper sulfate · $5H_2O$, 0.08 grams. Into this diet were incorporated 1 mg. of thiamine hydrochloride, 2 mg. of riboflavin, 1 mg. of pyridoxine hydrochloride, 4 mg. of calcium pantothenate, 2 mg. of niacin, 200 mg. of choline chloride, 0.001 mg. of biotin, and 0.4 mg. of 2-methyl-1,4-naphthoquinone. Twice weekly each rat received a supplement of 0.25 cc. of corn oil containing 2,000 units of vitamin A and 200 units of vitamin D (Natola) and once weekly 3 mg. of α-tocopherol in 0.03 cc. of ethyl laurate.