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The Influence of Nerve-Impulse Flow on the Synthesis and Metabolism of 5-Hydroxytryptamine

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The 5-hydroxytryptamine of the central nervous system appears to be almost entirely confined to special neurons, where it is largely localized in granules or synaptic vesicles of the terminal fibre system. Depolarization of the cell membrane of this system causes the release of 5-hydroxytryptamine into the extracellular space. The 5-hydroxytryptamine released is partly taken up again by the nerve terminal and part is deaminated by monoamine oxidase.

If the cell bodies are separated from their fibre systems by cutting the axons, the fibres degenerate, after a latency of a few days, as indicated by the disappearance of 5-hydroxytryptamine and its synthesizing enzymes. In the present studies we investigated the immediate effects, i.e. within 1 to 6 h, of cutting the axons, and thus interrupting the flow of impulses from the cell bodies to the terminal system, on 5-hydroxytryptamine synthesis and metabolism.

Methods for studying 5-hydroxytryptamine synthesis in vivo. It is generally believed that the first step in 5-hydroxytryptamine synthesis, i.e. the hydroxylation of tryptophan on the 5-position, is rate-limiting. Thus, if any physiological control of 5-hydroxytryptamine synthesis occurs, it presumably acts by changing the activity of tryptophan hydroxylase. Alternatively, since this enzyme is not saturated under normal conditions, the transport of the substrate might be controlled by physiological activity.

So far, only indirect methods have been available for investigating the rate of tryptophan hydroxylation *in vivo*. For example, endogenous 5-hydroxytryptamine and 5-hydroxyindol-3-ylacetate concentrations have been measured after inhibition of the enzymes (or mechanism) responsible for their formation or degradation (or efflux of 5-hydroxyindol-3-ylacetate out of the brain), or the appearance and disappearance of isotopically labelled 5-hydroxytryptamine have been studied after the administration of labelled tryptophan. In the present investigation we have measured the accumulation of the normally undetectable intermediate 5-hydroxytryptophan, induced by an inhibitor of 5-hydroxytryptophan decarboxylase. Preliminary observations suggest that this procedure yields a reliable measure of the rate of tryptophan hydroxylation. For example, the initial increase in 5-hydroxytryptophan concentration was found to be approximately linear. In agreement with earlier suggestions, the tryptophan hydroxylase of the mouse brain was found to be normally about half-saturated with respect to its substrate. Plotting the reciprocals of 5-hydroxytryptophan accumulation against those of substrate concentration in the brain yielded a straight line and, assuming a uniform concentration of tryptophan in the brain, a K_m value of 5×10^{-5} M was obtained. This K_m value is in reasonably good agreement with published data on partially purified enzyme *in vitro*.

By using this procedure the rate of 5-hydroxytryptophan formation in normal mouse brain was found to be about $0.4 \mu\text{g}$ (1.8 nmol)/h per g, the normal 5-hydroxytryptamine concentration being about $0.5 \mu\text{g}$ (2.8 nmol)/g in the same mouse population.

Effect of axotomy. The most clear-cut results have so far been obtained with the descendant spinal 5-hydroxytryptamine fibre system. Transection of the rat spinal cord caused a 40% decrease in the 5-hydroxytryptophan accumulation induced by a decarboxylase inhibitor [*N*-(2,3,4-trihydroxybenzyl)-*N'*-(DL-seryl)hydrazine, 800 mg/kg, or 3-hydroxybenzylhydrazine, 100 mg/kg intraperitoneally). The change appears to be immediate, since it was fully developed when the inhibitor was given simultaneously with the transection, analysis being performed 1 h later. Moreover, the effect was independent of the substrate concentration, varied by means of L-tryptophan administration. Since the effect was equally large even at tryptophan concentrations sufficient for saturation of tryptophan hydroxylase, a change in $V_{\max}$, rather than K_m appears to be involved. Studies by Kuhar *et al.* (1971) indicate that the half-life of tryptophan hydroxylase in the rat forebrain is about 2 days. The rapid decline in tryptophan hydroxylase activity observed in the present study can thus not be due to inhibition of enzyme formation. It might be speculated that in

the resting neuron part of the enzyme occurs in an inactive state, perhaps bound to the synaptic vesicles or cell membrane; just as cell membrane depolarization will induce release of transmitter from its storage sites, it may cause release also of the tryptophan hydroxylase, but in this case the release will occur into the cytoplasm rather than into the synaptic cleft. A mechanism of this type might, of course, involve a cofactor rather than the enzyme itself.

Another conclusion to be drawn from the present findings is that tryptophan hydroxylation appears to proceed at a fairly high rate even in the absence of an impulse flow. This phenomenon was even more striking in another experimental series involving the ascendant cerebral 5-hydroxytryptamine-carrying fibre system. In fact, axotomy, caused by a transverse cerebral hemisection, induced no detectable decrease in the 5-hydroxytryptophan accumulation on the lesioned side after decarboxylase inhibition, even though some indirect evidence suggested a slight decrease in turnover rate (Bédard *et al.*, 1972). The reason for the different outcome of the spinal and cerebral studies has not yet been elucidated. In any event, both studies emphasize the high rate of tryptophan hydroxylation occurring in the resting 5-hydroxytryptamine neuron. Since very little 5-hydroxytryptamine appears to be released from resting neurons, this would indicate the occurrence of a considerable 5-hydroxytryptamine turnover intraneuronally under these conditions. This invites the speculation that an important control mechanism depends on the degree of saturation of the intraneuronal storage sites. In the resting neuron a high saturation is reached, leading to increased intraneuronal deamination of newly synthesized 5-hydroxytryptamine. When 5-hydroxytryptamine is released by the nerve impulses, the extent of saturation of the storage sites is decreased, leading to increased uptake and diminished intraneuronal deamination.

We have also investigated the possible existence of a feedback regulation of the tryptophan hydroxylase. It was found in mice that treatment with a monoamine oxidase inhibitor (nialamide or pargyline) caused a decrease in the 5-hydroxytryptophan accumulation, induced by a decarboxylase inhibitor. The decrease was not observed when the inhibitors of the two enzymes were given simultaneously but only when the monoamine oxidase inhibitor was given beforehand, leading to an increased 5-hydroxytryptamine concentration. Also, the two hallucinogenic agents LSD-25 and dimethyltryptamine caused a decrease in the 5-hydroxytryptophan accumulation. These agents are believed to activate postsynaptic 5-hydroxytryptamine receptors. The data thus support the existence of a feedback mechanism operating via changes in 5-hydroxytryptamine concentrations and/or in the activity of postsynaptic 5-hydroxy-

tryptamine receptors. Changes in the rate of nerve-impulse flow may be involved in this case, too.

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Metabolic Regulation in the Release and Action of Excitatory and Inhibitory Amino Acids

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A compelling body of evidence supports the postulated role of γ -aminobutyrate as the inhibitory transmitter released at synapses in many different neuronal pathways in the mammalian brain and spinal cord; glycine probably exercises a similar function in the spinal cord (Curtis & Johnson, 1970; Curtis, 1971). Extracellular glutamate and aspartate depolarize central neurons, and numerous electrical and pharmacological comparisons have failed to distinguish between the mechanisms underlying these actions and those whereby unidentified natural excitatory transmitters mediate excitatory postsynaptic potentials (Curtis & Johnston, 1970; Curtis *et al.*, 1972). Concepts of metabolic regulation embracing a transmitter or otherwise 'modulatory' function of these four amino acids must take into account the metabolic correlates of their release, action and uptake.

Little is known of the factors controlling the release of synaptically active amino acids, but these would probably include the movements of ions associated with impulses arriving at axon terminals. Electrical stimulation or a high concentration of K^+ in the medium causes the release of both excitatory and inhibitory amino acids *in vitro* (Machiyama *et al.*, 1970; Hopkin & Neal, 1971; Arnfred & Hertz, 1971; de Belleruche & Bradford, 1972). Factors influencing glucose transport and oxidation, CO_2 fixation, the activity of amino acid-synthesizing enzymes and reuptake of the released transmitters and/or their metabolites must all be considered in relation to the regulation of transmitter synthesis. The claim by Kerpel-Fronius & Hajos (1971) that mitochondria in axon terminals are unable to oxidize α -oxoglutarate and succinate and the proposal that this apparent block in the tricarboxylic acid cycle is related to the synthesis and release of transmitter amino acids are pertinent to these considerations.

The action of excitatory amino acids *in vivo* is associated with an increase in the incorporation of ^{14}C from $[U-^{14}C]$ glucose into amino acids related to the tricarboxylic acid cycle (Watkins, 1971a). The