PROCEEDINGS OF THE BIOCHEMICAL SOCIETY

The 524th. Meeting of the Society was held at University College London, Gower Street, London WC1E 6BT, on Monday and Tuesday, 27 and 28 March 1972, when the following papers were presented:

SYMPOSIUM ON 'NEUROTRANSMITTERS AND METABOLIC REGULATION'

Neuronally Mediated Enzyme Induction

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Increased utilization of noradrenaline resulting from direct nerve stimulation or reflex increase in sympathetic discharge is accompanied by an immediate rise of catecholamine synthesis from tyrosine (Alousi & Weiner, 1966; Gordon *et al.*, 1966). This rapid regulation has been postulated to be the consequence of a decreased end-product inhibition of tyrosine hydroxylase by noradrenaline or an increased availability of cofactor and/or substrate at the enzyme site (Nagatsu *et al.*, 1964; Sedvall & Kopin, 1967). In any instance of this immediate adaptation to increased transmitter utilization a rise in the activity of tyrosine hydroxylase *in vitro* has been observed (Sedvall & Kopin, 1967; Dairman *et al.*, 1968). However, recent studies have shown that a prolonged increase in the activity of peripheral and central adrenergic neurons produce also a rise in the tyrosine hydroxylase activity *in vitro* (Mueller *et al.*, 1969a; Musacchio *et al.*, 1969). This increase in the activity of the rate-limiting enzyme of noradrenaline synthesis is neuronally mediated, since it can be abolished by transection of preganglionic sympathetic nerves or splanchnic fibres supplying the adrenal medulla (Thoenen *et al.*, 1969a,b). The fact that inhibitors of protein synthesis are able to block the increase in tyrosine hydroxylase suggests, together with enzyme kinetic data, that the rise in enzyme activity results from an augmented synthesis of enzyme protein (Mueller *et al.*, 1969b,c). The phase difference between the rise of tyrosine hydroxylase activity in stellate ganglia and the heart, occurring after prolonged increase in sympathetic reflex discharge, implies a transport of completed enzyme, or the message for its synthesis, from the cell body to the peripheral part of the adrenergic neuron (Thoenen *et al.*, 1970). In analogy to bacterial systems, in which the genetic information for enzymes involved in a given metabolic pathway is located in adjacent chromosomal areas, which form an operational unit, one could assume that the enzymes responsible for the synthesis of noradrenaline are arranged in a similar way. However, increased sympathetic activity provoked by cold exposure, immobilization stress

or administration of reserpine resulted in a rise of tyrosine hydroxylase and dopamine β -hydroxylase activities only (Mueller *et al.*, 1969a; Molinoff *et al.*, 1970; Kvetňanský *et al.*, 1971; Thoenen *et al.*, 1971), whereas that of the third enzyme involved in the synthesis of noradrenaline, dopa decarboxylase, remained unchanged (Thoenen *et al.*, 1971; Black *et al.*, 1971). It is noteworthy that dopa decarboxylase has a rather wide distribution and is not selectively located in adrenergic neurons and adrenal chromaffin cells as is the case for tyrosine hydroxylase and dopamine β -hydroxylase (Andén *et al.*, 1964; Klingman, 1965; Iversen *et al.*, 1966).

The trans-synaptic induction of enzymes involved in the synthesis of noradrenaline is not only of importance as a mechanism of long-term adaptation to increased transmitter utilization but is of more general interest, since it represents the regulation of the synthesis of a well-defined macromolecule in the terminal adrenergic neuron by the activity of the preganglionic cholinergic nerves. This mechanism of trans-synaptic regulation of protein synthesis could be of importance for the ontogenesis of organs and for the development of drug-addiction. In this context it is of interest that transection of the preganglionic cholinergic nerves of the superior cervical ganglia in newborn rats delays both general growth and differentiation of the terminal adrenergic neuron, and that the development of tolerance to morphine is accompanied by an increase in tyrosine hydroxylase and dopamine β -hydroxylase in the brain stem.

- Alousi, A. & Weiner, N. (1966) *Proc. Nat. Acad. Sci. U.S.* **56**, 1491
Andén, N. E., Magnusson, T. & Rosengren, E. (1964) *Experientia* **20**, 328
Black, I. B., Hendry, I. & Iversen, L. L. (1971) *Nature (London)* **231**, 27
Dairman, W., Gordon, R., Spector, S., Sjoerdsma, A. & Udenfriend, S. (1968) *Mol. Pharmacol.* **4**, 457
Gordon, R., Spector, S., Sjoerdsma, A. & Udenfriend, S. (1966) *J. Pharmacol. Exp. Ther.* **153**, 440
Iversen, L. L., Glowinski, J. & Axelrod, J. (1966) *J. Pharmacol. Exp. Ther.* **151**, 273
Klingman, G. L. (1965) *J. Pharmacol. Exp. Ther.* **148**, 14
Kvetňanský, R., Gewirtz, G. P., Weise, K. V. & Kopin, I. J. (1971) *Mol. Pharmacol.* **7**, 81
Molinoff, P. B., Brimijoin, St., Weinshilboum, R. & Axelrod, J. (1970) *Proc. Nat. Acad. Sci. U.S.* **66**, 453
Mueller, R. A., Thoenen, H. & Axelrod, J. (1969a) *Science* **163**, 468

- Mueller, R. A., Thoenen, H. & Axelrod, J. (1969b) *J. Pharmacol. Exp. Ther.* **169**, 74
- Mueller, R. A., Thoenen, H. & Axelrod, J. (1969c) *Mol. Pharmacol.* **4**, 457
- Musacchio, J. M., Julou, L., Kety, S. S. & Glowinski, J. (1969) *Proc. Nat. Acad. Sci.* **63**, 1117
- Nagatsu, T., Levitt, M. & Udenfriend, S. (1964) *J. Biol. Chem.* **239**, 2910
- Sedvall, G. C. & Kopin, I. J. (1967) *Biochem. Pharmacol.* **16**, 39
- Thoenen, H., Mueller, R. A. & Axelrod, J. (1969a) *Nature (London)* **221**, 1264
- Thoenen, H., Mueller, R. A. & Axelrod, J. (1969b) *J. Pharmacol. Exp. Ther.* **169**, 249
- Thoenen, H., Mueller, R. A. & Axelrod, J. (1970) *Proc. Nat. Acad. Sci. U.S.A.* **65**, 58
- Thoenen, H., Kettler, R., Burkard, W. & Saner, A. (1971) *Naunyn-Schmiedeberg Arch. Pharmacol.* **270**, 146

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