The Structure of the Metabolic Pathways Involved in the Metabolism of Neurotransmitters, with Emphasis on the γ -Aminobutyrate System

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The neurotransmitters acetylcholine, 5-hydroxytryptamine, dopamine, noradrenaline and γ -aminobutyrate are synthesized in the brain from precursors, entering the brain from the bloodstream. Schematically one can divide the metabolic pathways of these compounds into three broad categories, as follows.

(1) The neurotransmitter is part of a linear one-way pathway, in which the precursor is converted in a few steps into the transmitter and subsequently degraded to an end product leaving the brain. Apart from the initial precursor, none of the members of these pathways plays a significant role in other metabolic pathways in the brain. The interrelation of these pathways with other metabolic pathways consists only at the level of cofactors, ionic environment, pH etc.

(2) The transmitter is part of a similar system as to that in category (1), but some members of the pathway are utilized in other reactions and the degradation products of the neurotransmitter might be utilized again for the synthesis of the transmitter. The acetylcholine system is an example.

(3) The neurotransmitter is part of a complex set of metabolic reactions. Most of the members of these pathways are also members of other pathways. The γ -aminobutyrate system is an example. The part of the γ -aminobutyrate system that is specifically involved in the transmitter role of γ -aminobutyrate cannot be separated from other parts of the system.

The most important features of the γ -aminobutyrate system are (Van den Berg *et al.*, 1969; Van den Berg & Garfinkel, 1971) as follows.

(a) γ -Aminobutyrate is synthesized presynaptically from glutamate, originating from the tricarboxylic acid cycle.

(b) γ -Aminobutyrate is degraded 'postsynaptically' (neuronal cell bodies, dendrites and glia cells) to succinate, which enters the tricarboxylic acid cycle.

(c) The processes listed under (a) and (b) result in a transport of tricarboxylic acid-cycle intermediates from the presynaptic site to the 'postsynaptic' site. It can be argued that glutamine flows in the opposite direction, thereby compensating for the loss of intermediates in the presynaptic site.

Reuptake of γ -aminobutyrate into the nerve-endings from which it originates is probably quantitatively not very important unless the 'physiological'

turnover of γ -aminobutyrate is greater than the metabolic turnover ($0.14 \mu\text{mol/min per g}$).

The γ -aminobutyrate system is therefore not only part of a complex network of reactions, but it also couples the presynaptic and 'postsynaptic' structures together into one system. This type of metabolic coupling of the presynaptic and 'postsynaptic' structures does not occur in the systems classified under (1).

Van den Berg, C. J., & Garfinkel, D. (1971) *Biochem. J.* **123**, 211–218

Van den Berg, C. J., Krzalic, Lj., Mela, P. & Waelsch, H. (1969) *Biochem. J.* **113**, 281–290

The Effect of Electrical Stimulation of the Locus Coeruleus on the Endogenous Concentration of 4-Hydroxy-3-methoxyphenylethylene Glycol in Rat Brain

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Free 4-hydroxy-3-methoxyphenylethylene glycol and its sulphate ester have been shown (Mannarino *et al.*, 1963; Schanberg *et al.*, 1968a), by the use of radioisotope techniques, to be the major metabolites of noradrenaline in brain. 4-Hydroxy-3-methoxyphenylethylene glycol has been demonstrated by g.l.c. to be present in the brain of a number of species (Schanberg *et al.*, 1968b; Sharman, 1969). Histochemical studies (Ungerstedt, 1971) have shown noradrenaline-containing neurons originate in the locus coeruleus, a bilateral structure innervating all parts of cortex. Because of the effects of psychotropic drugs on these neurons (Graham & Aghajanian, 1971) and of the behavioural effects on its stimulation (Arbuthnott *et al.*, 1970), increasing attention has also been focused on this nucleus.

Male albino Wistar rats (180–220 g) were anaesthetized with 1.6% Halothane in O₂. A concentric bipolar electrode was placed stereotactically in the position of the left locus coeruleus, and electrical stimulation for 1 h was by 2 ms constant-current pulses, alternating in polarity at 0.2 mA and 10/s. Rats were then decapitated and the brains removed. Total 4-hydroxy-3-methoxyphenylethylene glycol was extracted from brain tissue by a method involving precipitation of brain protein with HClO₄, overnight enzymic hydrolysis of conjugated 4-hydroxy-3-methoxyphenylethylene glycol, ethyl acetate extraction at pH 5.0 and chromatography on Bio-Rad AG1 resin (X4; Cl⁻ form). The eluate was acetylated (Sharman, 1969), trifluoroacetylated (Bond, 1972) and

chromatographed on a Perkin Elmer 900 gas chromatograph with a ^3H -foil electron-capture detector. The mean concentration of total 4-hydroxy-3-methoxyphenylethylene glycol was found to be 95 (S.E.M. ± 9 ; 21 animals) ng/g for whole brain and 82 (S.E.M. ± 7 ; 13 animals) ng/g for cerebral cortex. Electrical stimulation of the left locus coeruleus for 1 h produced a significant ($P < 0.005$) increase in total 4-hydroxy-3-methoxyphenylethylene glycol of 70% in whole brain and a significant ($P < 0.0025$) increase of 60% in pooled left and right cerebral cortices. The separate analysis of left and right cerebral cortices suggested that the increase in 4-hydroxy-3-methoxyphenylethylene glycol was ipsilateral.

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- Arbuthnott, G. W., Crow, T. J. & Spear, P. J. (1970) *J. Physiol. (London)* **211**, 28P
 Bond, P. A. (1972) *Biochem. Med.* **6**, 36
 Graham, A. W. & Aghajanian, G. K. (1971) *Nature (London)* **234**, 100
 Mannarino, E., Kirshner, N. & Nashold, B. S. (1963) *J. Neurochem.* **10**, 373
 Schanberg, S. M., Schildkraut, J. J., Breese, G. R., & Kopin, I. J. (1968a) *Biochem. Pharmacol.* **17**, 247
 Schanberg, S. M., Breese, G. R., Schildkraut, J. J., Gordon, E. K. & Kopin, I. J. (1968b) *Biochem. Pharmacol.* **17**, 2006
 Sharman, D. F. (1969) *Brit. J. Pharmacol.* **36**, 523
 Ungerstedt, U. (1971) *Acta Physiol. Scand. Suppl.* 367

Effects of Diphenyleneiodonium and Trialkyltin Compounds on Photophosphorylation and Chloride-Hydroxide Exchange in Isolated Chloroplasts

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Work in this laboratory has shown that trialkyltin compounds mediate a Cl^- - OH^- exchange across mitochondrial and chloroplast membranes (Selwyn *et al.*, 1970; Watling & Selwyn, 1970). It has also been established that these compounds also produce a direct inhibition of phosphorylation in mitochondria (Aldridge & Street, 1964, 1971; Sone & Hagihara, 1964; Stockdale *et al.*, 1970). Under some conditions the Cl^- - OH^- exchange also produces, indirectly, inhibition of mitochondrial phosphorylation, respiration and adenosine triphosphatase activity (Coleman & Palmer, 1971; Dawson *et al.*, 1972; Selwyn *et al.*, 1972).

Holland & Sherratt (1971) have reported that

diphenyleneiodonium compounds mediate an anion- OH^- exchange across mitochondrial membranes but have little effect as direct inhibitors of mitochondrial phosphorylation. Observation of chloroplast volume changes, passive in NH_4Cl or on illumination in NaCl , shows that diphenyleneiodonium sulphate is effective in mediating a Cl^- - OH^- exchange across chloroplast membranes.

Kahn (1968) has reported that tributyltin is an inhibitor of photophosphorylation in chloroplasts from *Euglena* and spinach. In view of the complex effects found with mitochondria, the inhibition of photophosphorylation has been investigated in relation to the mediation of Cl^- - OH^- exchange.

Inhibition of photophosphorylation was estimated from the effect on these compounds on the stimulation by ADP of O_2 evolution from pea (*Pisum sativum*) chloroplasts at pH 7.6. Tributyltin and triphenyltin were most effective ($0.3\ \mu\text{M}$ and $0.6\ \mu\text{M}$ respectively), tripropyltin ($23\ \mu\text{M}$) and diphenyleneiodonium ($80\ \mu\text{M}$) were moderately effective, but trimethyltin ($0.3\ \text{mM}$) and triethyltin ($0.8\ \text{mM}$) were poor inhibitors. This order of effectiveness bears little relation to the order of effectiveness in mediating the Cl^- - OH^- exchange. It is also different from the pattern of inhibition of mitochondrial phosphorylation, where triethyltin, tripropyltin and tributyltin are all highly effective.

When electron transfer was uncoupled with NH_4Cl the trialkyltin compounds and diphenyleneiodonium sulphate inhibited O_2 evolution but only at concentrations much higher than those required to inhibit photophosphorylation. When carbonyl cyanide *m*-chlorophenylhydrazone was used as the uncoupler even higher concentrations of inhibitor were required, and in some cases no significant inhibition was observed at the highest usable concentration.

These observations confirm a direct inhibition of photophosphorylation by tributyltin, triphenyltin and tripropyltin, and the effectiveness of the butyl and phenyl compounds suggests a very hydrophobic region for the site of action.

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- Aldridge, W. N. & Street, B. W. (1964) *Biochem. J.* **91**, 287
 Aldridge, W. N. & Street, B. W. (1971) *Biochem. J.* **124**, 221
 Coleman, J. O. D. & Palmer, J. M. (1971) *Biochim. Biophys. Acta* **245**, 313
 Dawson, A. P., Dunnett, S. J. & Selwyn, M. J. (1972) *Biochem. J.* **127**, 67P
 Holland, P. C. & Sherratt, H. S. A. (1971) *Biochem. J.* **121**, 42P
 Kahn, J. S. (1968) *Biochim. Biophys. Acta* **153**, 203
 Selwyn, M. J., Dawson, A. P., Stockdale, M. & Gains, N. (1970) *Eur. J. Biochem.* **14**, 120
 Selwyn, M. J., Dunnett, S. J., Philo, R. D. & Dawson, A. P. (1972) *Biochem. J.* **127**, 66P