neurons (and not be buccal neurons) synthesized 5-hydroxy[^{14}C]tryptamine. It was estimated from a number of experiments that the GSC neurons *in vivo* form 0.8 ng of 5-hydroxytryptamine/2h at 20°C.

The capacity of the GSC neurons to metabolize [^{14}C]glucose and [^{14}C]glutamate *in vivo* was also investigated. Glucose was metabolized to form alanine, glutamine, glutamate and aspartate, but no γ -aminobutyrate. Histidine, arginine and five unknown substances were also formed. One of the unknown substances occurred in greater quantities than alanine, which is normally the predominant substance produced by the metabolism of glucose in snail nervous tissue *in vitro* (Osborne *et al.*, 1971). Electrical stimulation of the GSC neurons during perfusion with [^{14}C]glucose increased the production of some of the metabolites and also produced two further unidentified substances. Only three radioactive substances (glutamine, alanine and an unidentified compound), all in low concentrations, were detected in GSC neurons perfused with [^{14}C]glutamate. Electrical stimulation of the GSC neurons increased the concentrations of all these substances. These experiments demonstrated the chemical heterogeneity of neurons in the snail's central ganglia and also the chemical variation between the two 5-hydroxytryptamine-containing neurons in the snail *Helix pomatia* and the leech *Hirudo medicinalis*. Moreover, it was possible to assess the formation of 5-hydroxytryptamine *in vivo* and the effect of electrical stimulation on the metabolism of glucose and glutamate *in vivo* in a single neuron.

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Effect of Potassium Ion Concentration on Cultured Sympathetic Ganglia: Conclusions about Trans-synaptic Modulation

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Changes in trans-synaptic impulse traffic are capable of inducing long-term changes in the tyrosine hydroxylase activity of both sympathetic ganglia and adrenal medulla (Molinoff & Axelrod, 1971). However, the mechanism of such neurally mediated changes in enzyme activity is not yet understood.

We have examined the effect of raising the external K^+ concentration as a depolarizing stimulus on the tyrosine hydroxylase activity of adrenergic neurons of isolated mouse superior cervical ganglia maintained in organ culture. The ganglia were explanted from mice of from 2 to 28 days of age and maintained on membrane-filter rafts in modified Eagle's medium (see Mackay & Iversen, 1972) for up to 48 h. Choline acetyltransferase activity, a marker for presynaptic nerve remnants, had completely disappeared after this period in culture. Raising the K^+ concentration in the medium from 5.4 mM to 27, 54 or 81 mM for 48 h resulted in increases of up to 114% in tyrosine hydroxylase activity. Similar increases in the Na^+ concentration of the medium had no significant effect on this enzyme activity. The response was obtained both in the presence and in the absence of the nerve growth factor isolated from adult male mouse submaxillary glands. We have found the 7S fraction of nerve growth factor to be a potent stimulus for increasing tyrosine hydroxylase activity in neonatal superior cervical ganglia in culture, and when ganglia were stimulated maximally by 1.6 μg of nerve growth factor/ml they failed to respond further to increased K^+ . However, in the absence of added nerve growth factor K^+ evoked a 70% increase in tyrosine hydroxylase activity within 24 h.

The capacity of ganglia from very young animals to respond to a depolarizing stimulus may be important in the induction of enzyme activity in developing neurons. Black *et al.* (1971) observed a threefold increase in tyrosine hydroxylase activity in the mouse superior cervical ganglion between the 7th. and 13th. days of life. This increase correlated with the development of ganglionic synaptic junctions and could be prevented by decentralization.

Our present findings support the hypothesis that a trans-synaptic stimulus is responsible for the biochemical maturation of these ganglia, and furthermore suggest that increased depolarization of adrenergic neurons may be sufficient to account for the effects of presynaptic nerve traffic on post-synaptic activities. Work with cholinergic agonists

is at present in progress to investigate whether the effect of the presynaptic transmitter acetylcholine is mediated solely by depolarization or whether occupation of postsynaptic cholinergic receptors may provide some supplementary stimulus.

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Factors Influencing the Development of the Sympathetic Nervous System: Trans-synaptic Regulation and the Influence of Nerve Growth Factor

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There is evidence for trans-synaptic regulation of enzyme synthesis in the adrenergic neurons of the superior cervical ganglion. In the superior cervical ganglia of the adult rat the enzyme tyrosine hydroxylase responds to stress and various drug treatments by an increase in its activity (Thoenen *et al.*, 1969), and this response can be abolished by surgical transection of the preganglionic nerve trunk (Mueller *et al.*, 1969). We have shown in the adult mouse that decentralization causes a fall in the tyrosine hydroxylase activity in the superior cervical ganglia to 40% of control values after 10 days, and this effect of decentralization is reversible after 12 weeks.

In the neonatal mouse the normal development of tyrosine hydroxylase activity in the adrenergic neurons of the superior cervical ganglia is dependent on the formation of synaptic contact with preganglionic fibres (Black *et al.*, 1971). Section of the preganglionic nerve trunk in the 4-day-old mouse prevents the normal increase in tyrosine hydroxylase activity, which remains at about 40% of the normal adult value, and this effect persists for at least 12 weeks. This decentralization also prevents the normal increase in cell numbers in the superior cervical ganglia that occurs during the first 2 weeks of life.

Further evidence for the requirement of functional synaptic contact in the normal development of the adrenergic neuron comes from the present work. The ganglion-blocking drug pempidine blocks the depolarizing action of acetylcholine at ganglionic receptors. When this drug was given to neonatal mice during the critical period of the development of

the superior cervical ganglia it mimicked the effects of decentralization, preventing the normal increase in tyrosine hydroxylase activity, which rose only to 54% of the normal adult value.

The effects of nerve growth factor mimic those that occur during the normal development of the superior cervical ganglia; it causes an increase in cell numbers and an increase in the specific activity of tyrosine hydroxylase. To assess the role of nerve growth factor during the critical period of ganglion development, we examined the possibility that administration of nerve growth factor during this period could reverse the effects of decentralization or pempidine. It was found that administration of nerve growth factor stimulated tyrosine hydroxylase activity in superior cervical ganglia on both control and operated sides; however, the relative effect of the decentralization when compared with the control side remained unchanged. When large doses of nerve growth factor were administered tyrosine hydroxylase activity in the decentralized superior cervical ganglia was greater than that in the normal superior cervical ganglia of untreated controls, but if the treatment with nerve growth factor was stopped the enzyme activities of both the operated and control ganglia returned within 6 weeks to the same values as in animals who had received no treatment with nerve growth factor. Thus nerve growth factor was unable to reverse the long-term effects of decentralization on the developing neurons and was not able to induce the stability of the adult neuron to decentralization by itself. The differentiation of the neuron into a stable form would appear to require the acetylcholine-induced depolarization of this cell during its critical stage of development.

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