

## References

- HAAS, H.L. (1974). Histamine: action on single hypothalamic neurones. *Brain Research*, **76**, 363-366.
- RENAUD, L.P. & MARTIN, J.B. (1975a). Thyrotropin releasing hormone (TRH): depressant action on central neuronal activity. *Brain Research*, **85**, 150-154.
- RENAUD, L.P. & MARTIN, J.B. (1975b). Electrophysiological studies of connections of hypothalamic ventromedial nucleus neurons in the rat: evidence for a role in neuroendocrine regulation. *Brain Research*, **93**, 145-151.
- RENAUD, L.P., MARTIN, J.B. & BRAZEAU, P. (1975). Depressant action of TRH, LH-RH and somatostatin on activity of central neurons. *Nature*, **255**, 233-235.

## Failure of denervation to influence the high affinity uptake of choline by sympathetic ganglia

N.G. BOWERY\* & M.J. NEAL

*Department of Pharmacology, St. Thomas' Hospital Medical School, London, SE1 1EH and Department of Pharmacology, The School of Pharmacy, 29-39, Brunswick Square, London WC1N 1AX*

The transport process responsible for the uptake of choline in the central nervous system possesses high and low affinity components (Yamamura & Snyder, 1973; Haga & Noda, 1973; Neal & Gilroy, 1975) and there is some evidence that the high affinity choline transport process may be associated with cholinergic nerve terminals (Kuhar, Sethy, Roth & Aghajanian, 1973).

In the present study we have attempted to obtain further information on the possible association of the high affinity choline uptake process with cholinergic nerve endings by comparing the kinetics of choline uptake in normal and denervated ganglia.

Superior cervical ganglia were dissected from anaesthetized rats, de-sheathed, and then incubated for various times at 25°C in Krebs bicarbonate Ringer containing [<sup>3</sup>H]-choline (1 µCi/ml). The ganglia were then dissolved in Soluene (Packard) and the radioactivity determined by liquid scintillation counting. Unilateral decentralization of the ganglion was performed under halothane anaesthesia 12 to 17 days prior to excision of both ganglia.

Ganglia accumulated radioactivity when incubated with [<sup>3</sup>H]-choline ( $1.8 \times 10^{-7}$  M). This uptake was almost linear for 60 min at which time a tissue : medium ratio of approximately 9 was achieved. The effect of choline concentration on the uptake was studied over a concentration range of 0.2 to 600 µM. Since preliminary experiments indicated that uptake was linear over this concentration range for at least 20 min, incubations of this length were used to obtain estimates of the initial velocity of choline uptake. The kinetic data indicated that the uptake process for

choline in ganglia was saturable and, as in brain, could be resolved into a high and low affinity process, with apparent *K<sub>m</sub>* values of 2.1 µM and 43 µM respectively. The uptake of choline at both high and low concentrations was unaffected by denervation of the ganglia and a paired comparison of ganglia (7 pairs) at the lowest choline concentration (0.2 µM) revealed no significant difference between the choline uptake in denervated ganglia and the contralateral controls (Tissue; medium ratios =  $3.20 \pm 0.23$  and  $3.68 \pm 0.31$  respectively). It is unlikely that this result was due to failure of the denervation since ganglia were taken to be denervated only if there was obvious ptosis and a failure of the palpebral fissure to respond to bipolar stimulation (2 Hz, 0.5 ms, 30 V) of the preganglionic nerve stump. Denervated ganglia in the same series, showed greatly reduced ChAc activity. Furthermore, stimulation of denervated ganglia regionally superfused as described by Bowery & Tulett (1975) failed to increase the efflux of radioactivity from the soma although similar stimulation of control ganglia caused large increases in the release of radioactivity. These results imply that in ganglia the high affinity choline uptake process is not restricted to cholinergic nerve terminals.

## References

- BOWERY, N.G. & TULETT, D. (1975). A technique for the simultaneous measurement of effluent radioactivity and electrical responses in the rat superior cervical ganglia and its pre- and post-ganglionic trunks *in vitro*. *J. Physiol. (Lond)*, **246**, 20-21P.
- HAGA, T. & NODA, H. (1973). Choline uptake systems of rat brain synaptosomes. *Biochim. biophys. Acta*, **291**, 564-575.
- KUHAR, M.J., SETHY, V.H., ROTH, R.H. & AGHAJANIAN, G.K. (1973). Choline: Selective accumulation by central cholinergic neurones. *J. Neurochem.*, **20**, 581-593.
- NEAL, M.J. & GILROY, JILL (1975). High affinity choline transport in the isolated retina. *Brain Res.*, **93**. (In press).
- YAMAMURA, H.I. & SNYDER, S.H. (1973). High affinity transport of choline into synaptosomes of rat brain. *J. Neurochem.*, **21**, 1355-1374.