1,1-Dimethyl-3-acetoxypiperidine, a new cholinergic false transmitter

B.A. HEMSWORTH, S.M. SHREEVE & G.B.A. VEITCH

Department of Pharmacy, University of Aston, Birmingham B4 7ET.

The nicotinic action of 1,1-dimethyl-3-acetoxypiperidine, an analogue of acetylcholine, has been previously studied (Capetola, Gero & Zarro, 1975). This study is an investigation into the presynaptic action of 1,1-dimethyl-3-hydroxypiperidine, a cyclic analogue of choline in which the choline skeleton is immobilised by being fixed in a ring.

1,1-Dimethyl-3-hydroxypiperidine was found to be acetylated *in vitro* by choline acetyltransferase (ChAc) purified from rat brain homogenate. At 20 mM the analogue was acetylated 55% compared to choline 100%.

A crude preparation of synaptosomes was prepared from rat cerebral cortex (P_2 fraction of Gray & Whittaker, 1962) and incubated for 7 min at 37°C with [^{3}H]-choline (11 μM) and the amount of accumulated radioactivity measured. 1,1-Dimethyl-3-hydroxypiperidine was found to inhibit the high affinity uptake of choline, having an IC_{50} of 31 μM.

The P_2 fraction was incubated, as described above, with [^{14}C]-1,1-dimethyl-3-hydroxypiperidine (11–200 μM). Accumulation could be resolved into high and low affinity components, having K_T values of 7 μM and 194 μM respectively. K_T denotes that concentration of substrate which provides one-half maximal velocity of uptake.

In further experiments the synaptosomal metabolism of [^{14}C]-1,1-dimethyl-3-hydroxypiperidine was investigated. Synaptosomes were incubated with the labelled analogue (11 μM) and then extracted with a 1:10 dilution of electrophoresis buffer containing physostigmine (500 μM). The extract was freeze-dried and the residue dissolved in methanol. A mixture of 'cold' 1,1-dimethyl-3-hydroxypiperidine and 1,1-dimethyl-3-acetoxypiperidine was added to this methanol extract. The compounds now present in this extract were separated by paper electrophoresis for 2 h (Potter & Murphy, 1967) and visualised with iodine vapour. Bands corresponding to 1,1-dimethyl-3-hydroxypiperidine and 1,1-dimethyl-3-acetoxypiperidine were counted in a liquid scintillation counter. About 50% of the accumulated analogue was acetylated.

The phrenic nerve and endplate region of a mouse hemi-diaphragm was mounted in Krebs solution containing [^{14}C]-1,1-dimethyl-3-hydroxypiperidine 10 μCi, 30 μM) and physostigmine (30 μM) following the method of Potter, 1970. The phrenic nerve was stimulated at 10 Hz for 40 min at the voltage initially required for vigorous muscle twitching. The tissue was washed and then allowed to rest in fresh Krebs solution containing physostigmine (30 μM). After 10 min the nerve was stimulated at 5 Hz increasing to 10 Hz over a further 50 min period. The Krebs solution and the tissue were freeze-dried, and the tissue was extracted following the method of Potter, 1970. The compounds present were separated by paper electrophoresis and identified as described above. [^{14}C]-1,1-dimethyl-3-acetoxypiperidine was present in both the Krebs solution and the tissue extract.

These results suggest that cholinergic neurones accumulate 1,1-dimethyl-3-hydroxypiperidine by a high affinity uptake system. The analogue is then acetylated by ChAc to 1,1-dimethyl-3-acetoxypiperidine, which is released from the phrenic nerve on electrical stimulation.

References

CAPETOLA, R.J., GERO, A. & ZARRO, V.J. (1975). Study of two acetylcholine analogues with fixed conformations. *Archs. int. Pharmacodyn. Ther.*, **216**, 180–191.

GRAY, E.G. & WHITTAKER, V.P. (1962). The isolation of nerve endings from brain: an electron microscope study of cell fragments derived by homogenisation and centrifugation. *J. Anat., Lond.*, **96**, 79–88.

POTTER, L.T. (1970). Synthesis, storage and release of [^{14}C]-acetylcholine in isolated rat diaphragm muscles. *J. Physiol., Lond.*, **206**, 145–166.

POTTER, L.T. & MURPHY, W. (1967). Electrophoresis of acetylcholine, choline and related compounds. *Biochem. Pharmac.*, **16**, 1386–1388.