Dependence of potassium stimulated release of [^{3}H]-acetyl choline from the retina on high affinity choline uptake

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The retina possesses both a high and low affinity uptake process for choline, but only the high affinity process is sodium dependent and associated with a high degree of ACh formation (Neal & Gilroy, 1976).

In the present experiments we have studied the effect of potassium depolarization on the release of retinal ACh newly synthesized from [^{3}H]-choline. The incubation conditions employed during the uptake of [^{3}H]-choline by the retina were varied in an attempt to associate the high affinity uptake process with the potassium stimulated release of [^{3}H]-ACh.

Isolated rat retinae were incubated in Krebs Ringer bicarbonate containing [^{3}H]-choline (5×10^{-8}M) at 37°C for 30 minutes. The tissue was then washed and superfused in a small chamber at a rate of 1.2 ml/minute. The radioactivity released during 2 min periods was estimated by liquid scintillation counting. In some experiments, in which the medium contained eserine (30 μM) the [^{3}H]-choline and [^{3}H]-ACh were extracted and separated by high voltage paper electrophoresis before counting.

Depolarization with 50 mM KCl caused a fourfold rise in the efflux of radioactivity (3.84 ± 0.14, $n = 26$). This increased release was calcium dependent being only 1.6 times ± 0.06 ($n = 26$) the resting release in calcium free medium. Electrophoretic analysis of the superfusate before and during potassium stimulation indicated that the release of [^{3}H]-ACh increased by 21.8 ± 5.73, ($n = 7$) times, whilst the efflux of [^{3}H]-choline increased by only 2.0 ± 0.27 ($n = 6$). Thus the increase in the release of radioactivity caused by KCl was due primarily to an increase in ACh release.

When the retinae were loaded with [^{3}H]-choline in the absence of sodium ions or presence of hemicholinium-3 (10 μM) (procedures which inhibit the high affinity uptake of [^{3}H]-choline), there was, in the subsequent superfusion, a striking reduction in the potassium evoked release of radioactivity. Thus, the potassium evoked release after incubation in sodium free medium was 1.43 ± 0.05 ($n = 24$) times the resting release level, and in HC-3 treated retinae was 1.7 ± 0.03 ($n = 6$). These values represent an 85% and a 76% reduction in evoked release respectively, compared with potassium stimulated controls in normal medium.

These results suggest that potassium depolarization of retina causes [^{3}H]-ACh release from cholinergic neurones and are consistent with the suggestion that [^{3}H]-ACh is formed after uptake of [^{3}H]-choline by a high affinity, sodium dependent process.

S. Massey is an SRC student.

Reference

NEAL, M.J. & GILROY, J. (1975). High affinity choline transport in the isolated retina. *Brain Res.*, **93**, 548–551.