

The uptake and subcellular distribution of ^3H -choline by the retina

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It has been known for a long time that the vertebrate retina but not the optic nerve, contains acetylcholine (ACh) and possesses exceptionally high choline acetyltransferase (ChAc) activity (Feldberg & Mann, 1946; Hebb, 1963). Furthermore, it has been claimed that the retina has a higher binding capacity for dimethyl-d-tubocurarine than other areas of the cat central nervous system (De Robertis & Fiszer, 1968). Although these results strongly indicate a cholinergic mechanism in the retina, the location and function of the presumed cholinergic synapses is unknown.

In order to obtain further information on the retinal cholinergic system, we have examined the uptake and subcellular distribution of ^3H -choline by the retina.

Isolated rat or rabbit retinae were given a preliminary incubation in Krebs bicarbonate Ringer at 37°C for 10 minutes. ^3H -Choline was then added to give a final concentration of $6.5 \times 10^{-8}\text{M}$ and the incubations were continued for various times. The retinae were then recovered and washed. The accumulated radioactivity was estimated by liquid scintillation counting after dissolving the tissue in Soluene.

Rat and rabbit retinae rapidly accumulated radioactivity and after 45 min incubation, tissue/medium ratios of approximately six were achieved. Preliminary metabolic studies indicated that about 50% of the labelled choline was converted to ^3H -acetylcholine during 30 min incubation. Kinetic analysis of the uptake process suggested that it could be resolved into two components: a low affinity process (apparent

$K_m = 100\ \mu\text{M}$) and a high affinity process ($K_m = 2.15\ \mu\text{M}$). The high affinity uptake was temperature-dependent ($T/M = 0.7$ at 0°C) and was reduced by about 50% in the absence of sodium or by the presence of hemicholinium ($0.1\ \text{mM}$).

The subcellular distribution of the radioactivity accumulated by rabbit retinae incubated with a low concentration of ^3H -choline ($6.5 \times 10^{-8}\text{M}$) was studied on continuous sucrose density gradients (Neal & Atterwill, 1974). More than 80% of the radioactivity appeared to be localized in particles which had a median equilibrium density equivalent to 1.24 M sucrose. This 'bound' radioactivity was reduced by 50% following exposure of the particles to hypo-osmotic conditions. Over 85% of the ChAc activity measured on the same gradients was also present in particles which had the same equilibrium density as those accumulating labelled choline.

It is concluded that the retina possesses a high affinity uptake process for choline. This process is present in particles which may prove to be retinal cholinergic nerve endings.

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