Uptake of choline by rabbit corneal epithelium

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The corneal epithelium in most mammals has a content of acetylcholine (ACh) and choline acetyltransferase (ChAc) higher even than ganglion and brain, although its only nerve supply is sensory. ACh in this tissue appears to have no neural function (Stevenson & Wilson, 1974). We have, therefore, examined choline (Ch) uptake in the cornea to compare this property with that of other tissues with a high ACh synthesizing capacity.

The cornea, along with a few mm of attached sclera, was isolated from the rabbit eye. The endothelium was removed. The stromal layer with the intact epithelium was then incubated at 37°C in a holder which permitted both surfaces of the cornea to be bathed in separate media. Uptake of [^{3}H]-Ch (0.03–0.25 μCi contained in 250 μl of Krebs solution) was estimated by removing the epithelium at the end of the incubation and homogenizing in formic acid-acetone (15:85). Radioactivity in an aliquot of homogenate was counted using toluene-Triton-X100 scintillant. Ch and ACh in the remaining homogenate were separated by thin layer chromatography.

On application of Krebs containing [^{3}H]-Ch to the stromal side only, [^{3}H]-Ch appeared in the epithelium within 5 minutes. After 1 h, the radioactivity in the epithelium was ten times that remaining in the medium bathing the stromal side. On the other hand, bathing the epithelium in [^{3}H]-Ch resulted in no accumulation.

In subsequent experiments the uptake of [^{3}H]-Ch from the medium bathing the stromal side was studied over the concentration range 0.1–100 μM. Results were corrected for uptake at 0°C, which was taken to represent passive diffusion of [^{3}H]-Ch into the tissue. The data suggest the existence of a single uptake process showing Michaelis–Menten kinetics (apparent $K_m = 480$ μM and $V_{max} = 15$ nmol/mg protein h^{-1}). Uptake of [^{3}H]-Ch appeared to be unaffected by incubation with Na^+-free Krebs solution made isotonic with sucrose, although release of free Na^+ from stromal bound Na^+ could have obscured a low degree of Na^+ dependence. Uptake decreased by >50% in the presence of 1 mM dinitrophenol. Inclusion of 100 μM hemicholinium-3 (HC-3) in the incubation fluid caused only 50% inhibition of [^{3}H]-Ch uptake into the epithelium. The proportion of [^{3}H]-Ch recovered from the epithelium as [^{3}H]-ACh was 54 ± 11% (mean ± s.e. mean, $n = 14$) when the incubation was in 1 μM Ch, whereas at 100 μM, only 24 ± 4% ($n = 9$) was converted to [^{3}H]-ACh.

Ch uptake into the epithelium is similar to that found in the human placenta where an uptake with an apparent K_m of 364 μM is associated with 60% conversion of Ch to ACh after 20 min incubation (Welsch, 1976). Despite the large capacity of the epithelium for synthesizing ACh, the cornea appears to lack the high affinity choline uptake exhibited by cholinergic nerves (Yamamura & Snyder, 1973), which is characterized by Na^+ dependence and inhibition by low concentrations of HC-3.

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Antagonist affinity constants for adrenomedullary muscarinic receptors

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Muscarinic agonists have been shown to stimulate catecholamine release from the adrenal medulla (Critchley, Tibenham, Ungar, Waite & West, 1975). The action of these secretagogues is inhibited by muscarinic antagonists such as atropine. In view of the high concentrations of antagonist required to block the response, it was decided to investigate the affinity constants of a number of antagonists for the adrenal medullary receptors.

Canine adrenal glands were excised and perfused retrogradely with oxygenated Locke's solution at 37°C through the adrenolumbar vein at a constant