Since it was possible that the fractionation procedures might have caused destruction of acetylcholine-containing vesicles, in subsequent experiments only the nuclear fraction was removed (600 *g*, 20 min) prior to the assay of free and bound ACh. The loss of free ACh, determined without extraction with trichloroacetic acid, again accounted for most of the ACh lost during stimulation.

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Depletion of acetylcholine in the corneal epithelium

R. W. STEVENSON* and W. S. WILSON

Department of Pharmacology, University of Glasgow, Glasgow G12 8QQ

The mammalian corneal epithelium contains a very high concentration of acetylcholine (ACh), but its function in this tissue is unknown. Hemicholinium-induced depletion of rabbit epithelial ACh (to a level below 60% of the control) is accompanied by loss of the corneal reflex, suggesting involvement of ACh in sensory mediation (Fitzgerald & Cooper, 1971). We have re-examined this postulated association between epithelial ACh level and corneal reflex using the choline acetyltransferase (ChAc) inhibitor, *trans*-4-(1-naphthylvinyl) pyridine hydrochloride (NVP).

All experiments were performed in a darkroom under dim red light, since the active *trans* isomer of NVP photoisomerises readily to the less active *cis* isomer (White & Cavallito, 1970). Corneal epithelium of adult Dutch rabbits was scraped off under halothane/N_2O/O_2 anaesthesia and ACh measured by bioassay. The corneal reflex in the test and control eye was tested immediately before induction of anaesthesia (1) by touching the surface with a whisker (tactile) and (2) by exposing to a metered puff of ammonia vapour (painful).

Topical application of NVP in saline (at concentrations sufficient to produce maximum *in vitro* inhibition of ChAc) failed to reduce the ACh level of the test cornea below that of the contralateral control eye. Intraocular injection of NVP (producing concentrations in the aqueous humour within the range 5×10^{-5}M to $1{\cdot}2\times10^{-3}$M) resulted in a dose-dependent depletion of ACh in the corneal epithelium. The optimum time of action was 60 min and the lowest ACh level achieved (using $1{\cdot}2\times10^{-3}$M) was $37{\cdot}9\pm2{\cdot}3$% of the control. Neither doubling this dose nor repeating the injection 60 min later reduced ACh levels significantly further.

In all cases tested, rabbits, whose epithelial ACh content had been depleted in this way, possessed a corneal reflex to both painful and tactile stimuli. Thus, it seems that depletion of ACh levels in the epithelium below 40% does not *per se* abolish the corneal reflex.

When the cholinesterase (ChE) inhibitor, neostigmine, is topically applied to an untreated eye immediately before removal of the corneal epithelium, $24{\cdot}9\pm6{\cdot}2$% more ACh can be extracted. This ChE-labile pool of ACh may be an artifact or could represent a functionally distinct pool. When the ACh remaining in this ChE-labile pool was examined after intraocular NVP injection into both eyes, only $9{\cdot}5\pm3{\cdot}8$% more ACh could be extracted from the cornea to which neostigmine was applied. Thus, the relative proportions of the ChE-labile and ChE-resistant ACh pools were apparently different after NVP treatment.

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