Thus, at least three types of supersensitivity occur in the rat anococcygeus. The mechanism of the specific supersensitivity seems clear but the different types of non-specific supersensitivity produced by reserpine and thyroxine on the one hand and corticosterone and morphine on the other are unexplained and require further examination.

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The effect of electrical stimulation of the myenteric plexus-longitudinal muscle preparation of the guinea-pig ileum on its acetylcholine content

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The acetylcholine (ACh) content of the myenteric plexus-longitudinal muscle preparation was determined after extraction with trichloroacetic acid (Chang & Gaddum, 1933) by bioassay on the isolated guinea-pig ileum (Kosterlitz, Lydon & Watt, 1970). When such a preparation was incubated in Krebs solution containing choline (20 μM), the ACh content rose over a period of 30 min and was then maintained at a steady level for at least 240 min. In the presence of eserine (7·7 μM) the rate of rise was greater, the ACh content continued to increase slightly after 30 min and the final content was approximately double that in the absence of eserine.

After incubation for 70 min in the presence or absence of eserine, the tissue was homogenized in ice-cold 0·32 M sucrose solution containing eserine by means of a Teflon pestle—glass homogenizer and subsequently fractionated by differential centrifugation into nuclear (600 *g*, 20 min), mitochondrial (22,000 *g*, 30 min), microsomal (100,000 *g*, 60 min) and supernatant fractions. Comparisons of the fractions prepared from tissues incubated in the presence or absence of eserine showed that there was little difference between the ACh contents of the particulate fractions. In the supernatant fraction, however, eserine increased the ACh content by an average of 12 μg/g tissue, most of which was not protein-bound.

In a second series of experiments, preparations were pre-incubated for 70 min in the presence of eserine and then incubated for a further 60 min in the same solution either without stimulation or with supramaximal stimulation (1 ms pulse duration), at 0·017, 1 or 10 Hz. The ACh contents of the stimulated and unstimulated preparations were compared with the contents of tissues incubated for only 70 min. The spontaneous as well as the stimulated ACh outputs occurring during the second or main incubation period of 60 min were measured and the rate of synthesis of ACh determined. As far as ACh content is concerned there was no significant change at the lower frequencies but a decrease of 30% at 10 Hz. This decrease was nearly all accounted for by the ACh released during stimulation so that there appeared to be no synthesis of ACh during this period. Fractionation of the tissue showed that the decrease in the ACh content stimulation was due mainly to loss from the supernatant fraction.

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

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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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