Inhibition of the acetylcholine-destroying activity of the guinea-pig vas deferens by eserine or by dyflos

SIR,—The increase produced by eserine in the height of the responses of the guinea-pig isolated vas deferens to stimulation of the hypogastric nerve (Huković, 1961) has been reported by several workers (Boyd, Chang & Rand, 1960; Burn & Weetman, 1963; Ohlin & Strömblad, 1963; Della Bella, Benelli & Gandini, 1964), but measurements of the inhibition of cholinesterase activity by the concentrations of eserine used appear to have been made only by Ohlin & Strömblad (1963), who used the Warburg manometric method with acetylcholine as substrate.

When the vas deferens is contracted by transmural stimulation of post-ganglionic nerves (Birmingham & Wilson, 1963) the height of the response is increased by eserine (10^{-6} g/ml) or by di-isopropylphosphorofluoridate (dyflos, 10^{-5} g/ml) (Birmingham, 1964, communication to British Pharmacological Society).

Experiments have now been made to measure the degree of inhibition of the acetylcholine-destroying activity of the vas deferens produced by 20 min exposure to 10^{-6} g/ml eserine or to 10^{-5} g/ml dyflos. The method used was similar to that

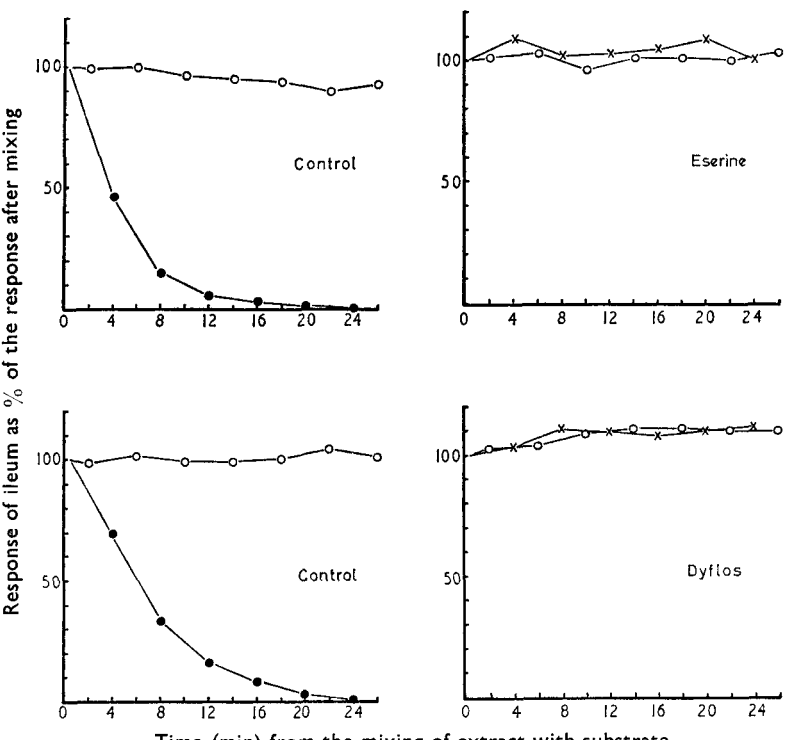

FIG. 1. Graphs of the mean responses of the guinea-pig ileum assay preparations to 0·15 ml samples from the reaction mixtures of the untreated controls —●—, the drug-treated extracts —x—, and the standard acetylcholine solution in Krebs —o—. The assay preparation was 3 cm of guinea-pig proximal ileum in 15 ml of Krebs solution at 37° bubbled with 95% oxygen, 5% carbon dioxide.

described by Boyd & others (1960) for their investigation of anticholinesterase activity in some anti-adrenaline drugs, and was chosen instead of the manometric method because it measures more directly the effect of the inhibiting drug and much lower substrate concentrations can be used. Drug concentrations are expressed in terms of the base.

For the four determinations made for each drug the enzyme mixture was obtained by grinding the vasa deferentia from two guinea-pigs with sand in a mortar containing 16 ml of Krebs solution. The supernatant part of this extract was decanted into a test tube kept in iced water. Aliquots of 2 ml were used as a source of acetylcholine-destroying enzyme and were either untreated, as controls, or treated with eserine (10^{-6} g/ml for 20 min) or dyflos (10^{-5} g/ml for 20 min) before being mixed with the 2 ml of Krebs solution containing the acetylcholine substrate. The final concentration of acetylcholine in the 4 ml of reaction mixture was 1×10^{-6} g/ml.

The reaction mixture was kept at 32° and the acetylcholine content of 0·15 ml samples was assayed at 4 min intervals on a preparation of guinea-pig ileum in 15 ml of Krebs solution at 37°. Samples from the reaction mixture were alternated with similar volumes from a standard acetylcholine solution in Krebs kept in the 32° water-bath.

The results are shown graphically in Fig. 1. Each point on the graphs is the mean of four determinations. The control graphs on the left show that the untreated enzymes mixtures gradually destroyed the acetylcholine substrate until at 24 min the samples from the reaction mixture failed to elicit any contraction from the ileum. The responses of the ileum to the standard doses of acetylcholine in Krebs kept under the same conditions were not reduced during the same period. The graphs on the right show the results obtained with eserine (upper graph) and with dyflos (lower graph). The acetylcholine concentration in the reaction mixtures did not diminish during the period of examination.

It is concluded from these results that a concentration of 1×10^{-6} g/ml of eserine or 1×10^{-5} g/ml of dyflos acting for 20 min inhibits completely the acetylcholine-destroying property of the guinea-pig vas deferens; this inhibition may account for the ability of eserine or dyflos to increase the height of the contractions of the vas deferens stimulated preganglionically or postganglionically.

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References

Birmingham, A. T. & Wilson, A. B. (1963). *Brit. J. Pharmacol.*, **21**, 569–580.
Boyd, H., Chang, V. & Rand, M. J. (1960). *Ibid.*, **15**, 525–531.
Burn, J. H. & Weetman, D. F. (1963). *Ibid.*, **20**, 74–82.
Della Bella, D., Benelli, G & Gandini, A. (1964). *J. Pharm. Pharmacol.*, **16**, 779–787.
Huković, S. (1961). *Brit. J. Pharmacol.*, **16**, 188–194.
Ohlin, P. & Strömblad, B. C. R. (1963). *Ibid.*, **20**, 299–306.