pP_2 of eserine and neostigmine on acetylcholine responses of isolated ileum

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pP_2 values of 7·9 and 8·0 for eserine and neostigmine show that these compounds are respectively about 200 and 300 times more potent in potentiating acetylcholine (ACh) responses on isolated cavy ileum than was previously found on frog rectus muscle. The increased tone and spontaneity usually produced by eserine and neostigmine were not prevented by morphine (10 to 100 μg/ml), which itself potentiated both ACh and histamine responses.

Edge (1967) determined the acetylcholine (ACh) potentiating potency of four anticholinesterases on frog isolated rectus abdominis muscle. This muscle was chosen because, unlike smooth muscle preparations, ACh-potentiating doses of anticholinesterases do not increase the resting tone. This paper reports the pP_2 values of eserine and neostigmine obtained on isolated cavy ileum.

Methods.—2–3 cm lengths of cavy ileum oral to the terminal lymph gland were suspended in a 5 ml bath of Tyrode solution bubbled with 5%CO_2 in O_2 at 37° C. Segments awaiting use were kept similarly. Volumes of 0·05–0·30 ml of agonist solution added to the bath were left for 30 s before replacing the bath fluid by a 5–6 s overflow. A 3 min cycle was used and when approximately equal responses (40–60% of maximum) were obtained, the anticholinesterase was introduced 2 min before the next addition of a one-half dose of ACh and replaced immediately after each overflow wash for a total of 12 cycles (35 min). This was sufficient time for equilibrium conditions to pertain. Adjacent segments of ileum were usually used for each interpolated pP_2 estimation. Four and five separate estimates were obtained for eserine and neostigmine, respectively, one of the estimates for neostigmine being obtained on a single ileal segment.

Drug concentrations are expressed as the salt. The compounds used were acetylcholine chloride (ACh), eserine sulphate, histamine dihydrogen phosphate, morphine sulphate and neostigmine methylsulphate.

Results.—Figure 1 shows one of the experiments with each compound. The mean 35 min pP_2 values were: neostigmine, $7{\cdot}97 \pm 0{\cdot}05$; eserine, $7{\cdot}86 \pm 0{\cdot}29$. Further pP_2 estimations were prevented by the spontaneity and tonal increase produced by either anticholinesterase.

Effect of morphine. Paton (1957) used morphine to inhibit the release of ACh from ileum in the presence of an anticholinesterase. Two of the pP_2 estimations of eserine, one of which is shown in Fig. 1, were obtained in the presence of morphine, 10 μg/ml. However, morphine did not prevent spontaneity, tonal increase or mucus expulsion which occurred in three of the four ileal segments used (Fig. 1d).

Morphine alone also caused an increase in the response to a constant dose of ACh. This potentiation was slight with 10 μg/ml but more marked with 100 μg/ml. These concentrations sometimes caused graded increases in rhythmic movements. The effects were quickly reversed following washout.

The potentiating action of morphine was not ACh-specific. A similar potentiation of histamine responses (Paton, 1957) was also observed.

Discussion.—The pP_2 values obtained for eserine and neostigmine confirm that these compounds are more effective (200 and 300 times) on this preparation than on amphibian skeletal muscle (Edge, 1967). Although morphine slightly potentiated ACh responses, the pP_2 values for eserine obtained in the presence of morphine were not greater than those obtained in its absence.

That anticholinesterases increase spontaneity and tone in various smooth muscle preparations is well known. Burn (1952) showed the effect in rabbit duodenum is glucose dependent. Paton (1957) found that morphine reduced the resting release from cholinergic nerve endings in cavy ileum and "quietened" the preparation. In the present experiments morphine tended to increase spontaneity. Carlyle (1963) attributed increase in tone of cavy trachealis muscle to stimulation of ACh release from nerve endings, an action of

eserine also postulated in the toad bladder (Bell, 1966) and superior cervical ganglion (Takeshige & Volle, 1962). Other suggested modes of action include a direct muscarinic action in the ileum (Harry, 1962) and a non-muscarinic intracellular site in chick amnion (Cuthbert, 1962) and toad bladder (Bell & Burnstock, 1965). By pharmacological depletion or inactivation of the cholinergic nerve endings it might be possible to decide the additional site of action of these anticholinesterases.

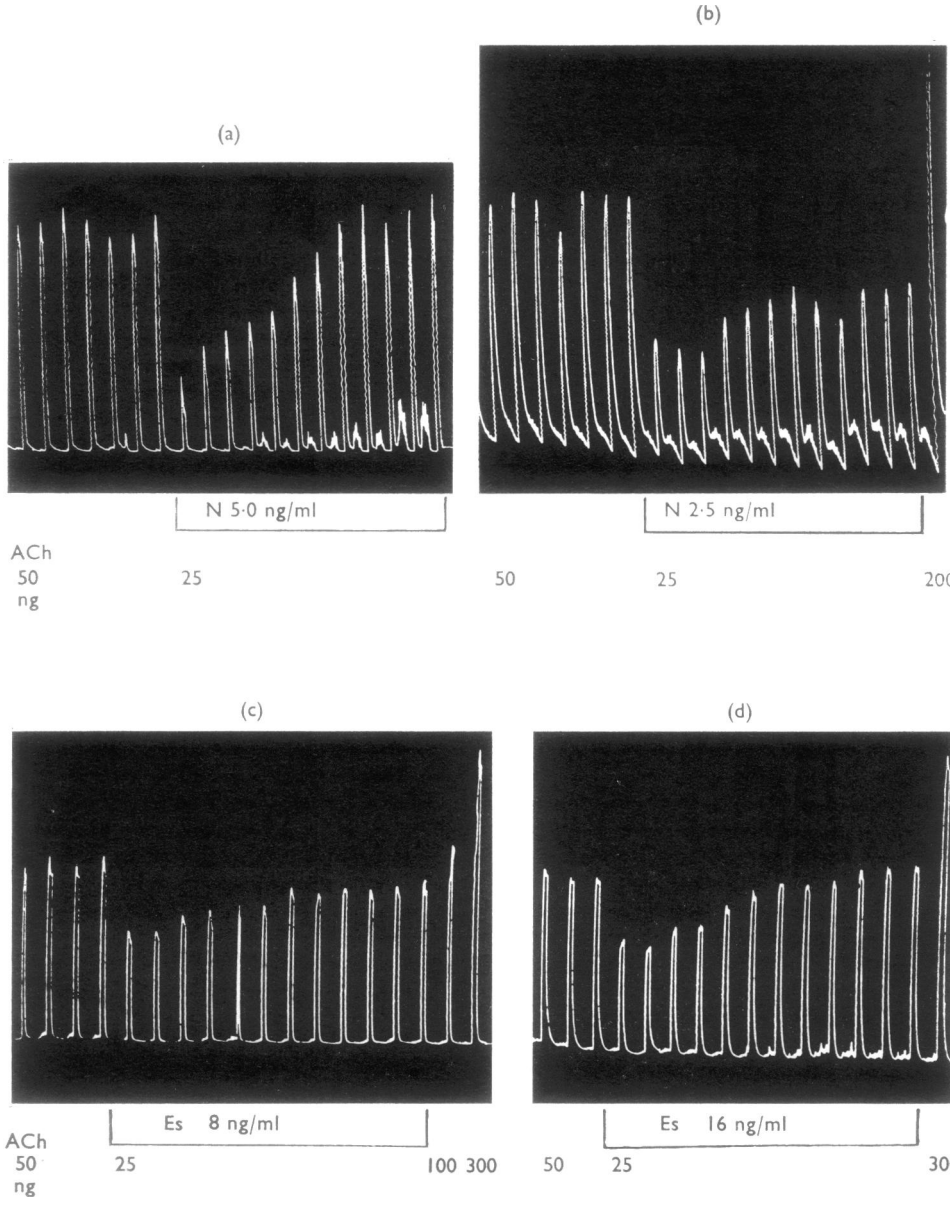

FIG. 1. Isolated ileal segments (two cavies) in 5 ml bath. Effect of neostigmine (N) (top) and eserine (Es) (bottom) on acetylcholine (ACh) contractions induced for 30 s every 3 min. ACh doses shown only when changed. In (c) and (d), Tyrode solution contained morphine (10 μg/ml). At —, the anticholinesterase was added 2 min before subsequent addition of ACh and replaced after each wash.

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