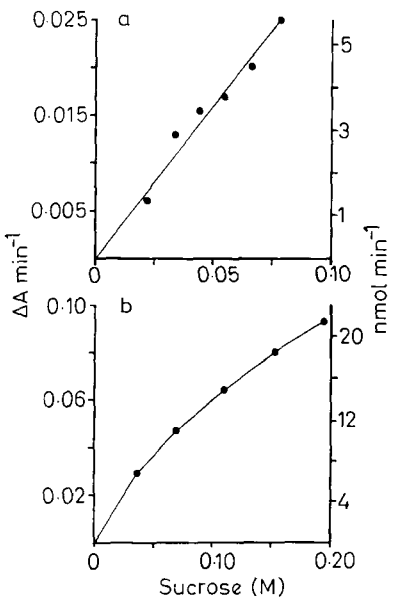

FIG. 1. The effect of sucrose concentration on the rate of non-enzymic breakdown of the substrates used to assay AChE and BuChE by the Ellman method. Incubation mixtures containing either (a) acetylthiocholine iodide (2·5 mM), or (b) butyrylthiocholine iodide (10 mM) were incubated (37°; pH 8·0) with different concentrations of sucrose, ranging from (a) 0–0·08 M, or (b) 0–0·2 M (final concentrations in cuvette). The initial rates of change of absorbance ($\Delta A\ min^{-1}$) at 412 nm were then determined spectrophotometrically. Values are corrected for buffer-mediated hydrolysis of the substrates, and each is the mean of duplicate determinations. Reaction rates are also given as nmol min^{-1} substrate utilized.

pure AChE and BuChE, that high concentrations of sucrose had no effect on enzyme-catalysed hydrolysis of these thioesters after correcting for its effect on their non-enzymic breakdown.

These results show, therefore that the presence of sucrose in the cholinesterase reaction mixtures can give rise to high non-enzymic rates of reaction. As the sucrose concentration is increased the rate of substrate utilisation increases. This is presumably because the sucrose molecule itself is acylated by the substrates. Similar findings have recently been reported by Hebb, Mann & Mead (1975) in the radiometric assay of choline acetyltransferase (ChAc). In this instance sucrose appeared to be acetylated non-enzymically by one of the substrates, acetyl-CoA, giving rise to misleadingly high estimates of enzyme activity. Acetylation of sucrose is also a possibility in cholinesterase assays employing acetylcholine as a substrate (e.g. pH-stat, radiometric, Michel, Hestrin methods; see Holmstedt, 1971).

The present findings suggest, therefore that adequate controls should be carried out when determining the activity of cholinesterases in subcellular fractions, or other preparations containing sucrose, by the spectrophotometric Ellman procedure. This is especially important when measuring low levels of enzyme activity in the presence of high sucrose concentrations. Removal of the sucrose by dialysis or gel-filtration, or simply running parallel blanks containing concentrations of sucrose equivalent to those present in the enzyme reaction mixtures, are possible ways of overcoming this problem.

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Bradykinin relaxes contracted airways through prostaglandin production

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Bradykinin is a potent constrictor of guinea-pig trachea and bronchus (Collier, Holgate & others, 1960). Paradoxically there have been a few reports where bradykinin has been shown to relax the trachea of guinea-pig (Ramos, Ramos & others, 1965; Iorio & Constantine, 1969), cat (Turker & Kiran, 1965; Turker & Ercan, 1976), dog (Turker & Khairallah, 1969) and rabbit (Fleisch & Calkins, 1976); and the bronchus of man (Mathé, Aström & Persson, 1971). Bradykinin has been reported to release prostaglandins (Damas & Deby, 1976; Erdos, 1976) and bradykinin-induced relaxation of cat trachea is blocked by aspirin (Turker & Ercan, 1976).

In a recent study in this laboratory, bradykinin (10^{-7} to 10^{-5}M) was found to contract trachea and bronchi of horse, ferret and guinea-pig. Bradykinin (10^{-6} to 10^{-5}M) relaxed the carbachol-contracted trachea and bronchi of dog, cat and rabbit, and the trachea, but not

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the bronchus, of guinea-pig. It seemed of interest to investigate whether PG production was involved in the tracheobronchial relaxant effects of bradykinin by using the potent PG-synthetase inhibitor, indomethacin (Vane, 1971).

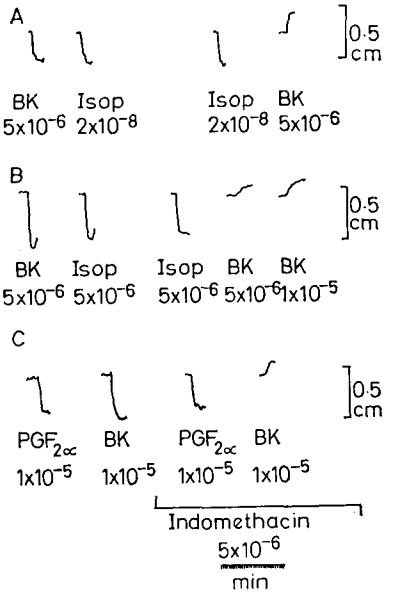

FIG. 1. Isotonic responses of airway smooth muscle (A—guinea-pig trachea; B—rabbit trachea; C—dog primary bronchus) in Krebs solution mixed with 5% CO_2 in O_2 at 37°. Resting tension = 3 g. Relaxations to bradykinin (BK), isoprenaline (Isop) and $PGF_{2\alpha}$ are taken from partial (50 to 60%) carbachol contraction. After indomethacin (5×10^{-6}) (present between the arrows in all preparations) responses to isoprenaline and $PGF_{2\alpha}$ were unchanged while bradykinin-induced relaxations were converted to small contractions. $PGF_{2\alpha}$ was chosen to show its action as a bronchorelaxant in these conditions in dog.

Drug doses are in molar bath concentration. Time marker indicates minutes. The vertical scale indicates actual change in length of the tissue in the bath—the pen tracing having an overall magnification of 8.

Tissue acquisition and preparation were similar to those established earlier (Eyre, 1973). Tracheal and bronchial strips from the same guinea-pig, rabbit or dog were mounted in pairs in separate organ baths under a resting tension of 3 g. Dose-response curves (isotonic contractions) to histamine, carbachol and bradykinin were established together with relaxation dose-response curves to isoprenaline, PGE_1, E_2 and $F_{2\alpha}$ and bradykinin measured on tissues partially (50–60%) contracted by carbachol. One strip of each pair was treated with indomethacin (5×10^{-6}M), which produced gradual lowering of the resting tone of varying magnitude, depending upon the tissue and the species. After 30 min, dose-response curves to several agonists were re-established, and the response of the indomethacin-treated tissue was compared with the control. Indomethacin potentiated the contractions due to histamine or carbachol but inhibited or reversed the relaxant effects of bradykinin without interfering with the responses of isoprenaline or prostaglandins.

That indomethacin relaxes the resting tone of the muscle and potentiates airway contractile responses to histamine and carbachol, confirms the findings of Orehek, Douglas & Bouhuys (1975). The blockade of relaxant effects of bradykinin by indomethacin is in agreement with the earlier observation with aspirin (Turker & Ercan, 1976). It may be suggested from this study that effects of bradykinin on airway smooth muscles depend upon the initial tone (Iorio & Constantine, 1969). Secondly bradykinin appears to release PG(s) from contracted airway smooth muscles. These prostaglandins in turn may cause airway relaxation.

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