Effects of two cholinesterase inhibitors on acetylcholine release from the guinea-pig isolated ileum preparation

In studies measuring the release of acetylcholine from the guinea-pig isolated ileum preparation, Schnieden & Weston (1969) used *NN'*-di-isopropylphosphorodiamidic fluoride (mipafox) to protect acetylcholine from hydrolysis by cholinesterases of the ileum. These workers found that the values for acetylcholine release in the presence of mipafox were very much lower than those obtained by other workers using eserine.

During the present investigations, values for acetylcholine release have been obtained under similar experimental conditions after treatment of the ileum with either eserine or mipafox. Segments of ileum, 3–4 cm long, were suspended in 10 ml organ baths containing Tyrode solution bubbled with oxygen and maintained at $37 \pm 1°$. The initial resting tension imposed on the ileum was 1 g. The preparation was washed at 10 min intervals for 1 h before exposure to either eserine or mipafox.

In the first series of experiments, the ileum was bathed in Tyrode containing eserine, 10 μg/ml, for 30 min, the bath fluid being changed at 10 min intervals. At the end of the next 10 min interval, the bath fluid was withdrawn for assay of acetylcholine content. When the irreversible cholinesterase inhibitor, mipafox, was used in the second series, the ileum was soaked in Tyrode containing mipafox, 10 μg/ml, for 75 min and washed at 10 min intervals for 1 h. 10 min later, the bath fluid was withdrawn for acetylcholine assay. In a third series of experiments, the ability of both eserine and mipafox to protect a dose of acetylcholine added to the ileum was assessed. The ileum was pretreated with either eserine or mipafox and the resting acetylcholine release determined as described above. Acetylcholine (30 ng) was then added to the bath and 10 min later a sample was withdrawn for assay. The value for the resting release was subtracted from the value obtained for resting + added acetylcholine.

The acetylcholine content of the bath fluid was assayed on the leech dorsal muscle preparation suspended in diluted Tyrode solution containing eserine and morphine (Murnaghan, 1958) and bubbled with oxygen. The active substance in the bath fluid was identified as acetylcholine in the following manner: (i) there was no significant difference between the results of parallel assays on the leech dorsal muscle and guinea-pig isolated ileum preparations; (ii) the activity of samples for assay was inhibited by tubocurarine on the leech dorsal muscle and by atropine on the guinea-pig ileum; (iii) loss of activity of samples occurred after heating in alkaline solution and subsequent neutralization.

The release of acetylcholine from the ileum was expressed as ng/g wet weight ileum in 10 min. In the presence of eserine, the rate of acetylcholine accumulation in 10 min was 646 ng/g (17 experiments). When mipafox was used, the value was 168 ng/g (16 experiments). When these results were compared using the Mann Whitney "U" test they were found to be significantly different ($P < 0{\cdot}001$). The percentage of acetylcholine added that could be accounted for in the bath fluid after 10 min was 94% in the presence of eserine (5 experiments) and 138% after mipafox treatment (6 experiments).

Table 1 compares the results obtained above with those taken from the publications of other workers who have used eserine or mipafox. Although there are differences in the periods over which acetylcholine collections were made, and in some instances differences in the concentrations of inhibitor used, it is clear that the values for acetylcholine release can be divided into two groups—high release in the presence of eserine and low release after treatment with mipafox.

This difference is difficult to explain. Eserine may produce an increased concentration of acetylcholine in the bath fluid by a mechanism different from its anti-

Table 1. *Published results for acetylcholine release from guinea-pig ileum treated with either eserine or mipafox*

Inhibitor concentration g/ml	Reported acetylcholine release calculated as ng acetylcholine/g ileum in 10 min	Reference
Eserine 2×10^{-6}	560	Paton & Zar, (1968)
Eserine 10^{-5}	1730	Schaumann, (1957)
Eserine 10^{-5}	833	Ogura, Mori & Watanabe, (1966)
Eserine 10^{-5}	646	Present study
Mipafox 10^{-5}	54	Johnson, (1963)
Mipafox 10^{-5}	62	Schnieden & Weston, (1969)
Mipafox 10^{-5}	168	Present study

cholinesterase action. Other actions for eserine have been reported (Carlyle, 1963; Werner & Kuperman, 1963). It is possible that mipafox may be interfering with the release of acetylcholine from the ileum, although no evidence for such an action has been reported. A third possibility is that mipafox, which is known to inhibit cholinesterase more effectively than acetylcholinesterase (Aldridge, 1953), does not give complete protection to endogenously released acetylcholine. Eserine, however, an inhibitor of both cholinesterase and acetylcholinesterase (Augustinsson, 1948), is able to give complete protection to endogenous acetylcholine. This suggests that there must be differences between the hydrolysis of endogenous and exogenous acetylcholine by cholinesterase since both eserine and mipafox gave full protection to a standard dose of acetylcholine added to the bath.

Ambache, Freeman & Hobbiger (1969) have provided support for this hypothesis. They showed that the acetylcholinesterase activity of the guinea-pig ileum is mainly localized in Auerbach's plexus whereas that of butyrylcholinesterase is largely associated with the longitudinal smooth muscle layer.

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