EFFECT OF CHOLINESTERASE INHIBITORS AND PAM ON THE RELEASE OF ACETYLCHOLINE FROM ISOLATED GUINEAPIG ILEUM

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Received for publication March 17, 1971

Eserine and mipafox have often been used as anticholinesterases to allow measurement of spontaneous release of acetylcholine from the ileum (1–4). Mipafox was chosen because of its apparent inability to release acetylcholine from smooth muscle (5). It has also been reported that values for acetylcholine release in the presence of mipafox (1) were much lower than those obtained by other workers using eserine (2, 3). It, therefore, follows that the choice of a particular anticholinesterase may influence the release of acetylcholine from smooth muscle. In order to experimentally test this possibility, we examined the effect of four cholinesterase inhibitors—eserine, prostigmine, phosphamidon and mipafox, on the spontaneous release of acetylcholine from isolated guineapig ileum.

PAM (2-pyridyl-aldoxime methiodide) reactivates the phosphorylated (inhibited) cholinesterase (6, 7); at certain concentrations, it induces the release of acetylcholine while at others, it inhibits the acetylcholine release from rat phrenic nerve diaphragm preparation (8). The effect of PAM on the release of acetylcholine from smooth muscle has so far not been studied. The effect of PAM, if any, on the acetylcholine output from isolated guineapig ileum, in the presence of anticholinesterases, has also been examined in the present study.

METHODS

Guineapigs (300–400 g) were killed by a blow on the neck and bled. The ileum was taken out, emptied of its contents and divided into several equal pieces. Two pieces, 3–4 cm long, from different parts of the intestine were combined into one sample to reduce the effect of the variations in choline acetylase content (9) along the length of the intestine. The pieces were tied off at both ends to prevent the mucus in the lumen from oozing into the bath fluid. The preparation was set up in the usual way in a 10 ml organ bath containing Tyrode solution at 37°C with a resting tension of 1 g. The preparation was washed twice at 10 minutes intervals before exposure to any drug. The preparation was next bathed in Tyrode containing the cholinesterase inhibitor for 30 minutes, the bath fluid being changed at 10 minute intervals; at the end of next 15 minutes, the bath fluid was withdrawn for the assay of acetylcholine. In another series of experiments, PAM was added in the bath 15 minutes before the collection of test sample.

The biological assay for acetylcholine was made on a fresh piece of isolated guinea-
pig ileum on the same day as, and immediately after, collection of the test sample. Known concentrations of drugs were added to the bath before assay to allow for a possible sensitization of the tissue to acetylcholine. The identification of the active substance in the bath fluid as acetylcholine was based on the following criterion. The spasmogenic activity was destroyed by boiling with sodium hydroxide solution; The activity of the extracts was abolished by atropine. The release of acetylcholine was expressed as ng/g wet weight of ileum.

The drugs employed in this study include eserine, prostigmine, mipafox (N, N'-di-isopropyl phosphorodiamidic fluoride), phosphamidon (0-[2-chloro-2-(diethylcarbamoyl)-1-methyl-vinyl]-0, 0-dimethyl phosphate), PAM (2-pyridyl-aldoxime methiodide) and atropine sulphate.

RESULTS

1) Effect of eserine, prostigmine, phosphamidon and mipafox on the release of acetylcholine from guineapig ileum.

The accumulation of acetylcholine in 15 minutes with eserine, prostigmine, phosphamidon and mipafox (each $1 \times 10^{-5}$ g/ml) was 694, 478, 227 and 151 ng/g respectively (Table 1).

| S.N. | Drugs         | Concentration g/ml | Acetylcholine release (ng/g ileum) in 15 min | A | In the absence of PAM | P (P) | B | In the presence of PAM |
|------|---------------|-------------------|---------------------------------------------|---|-----------------------|-------|---|-----------------------|
| (1)  | Eserine       | $1 \times 10^{-5}$ | 694 (14)                                   | <0.01 | 581 (12)                  | <0.01 | 503 (12) | <0.01 |
| (2)  | Prostigmine   | $1 \times 10^{-5}$ | 478 (12)                                   | <0.01 | 398 (10)                  | <0.01 | 341 (10) | <0.01 |
| (3)  | Phosphamidon  | $1 \times 10^{-5}$ | 227 (12)                                   | <0.01 | 189 (11)                  | <0.01 | 167 (10) | <0.01 |
| (4)  | Mipafox       | $1 \times 10^{-5}$ | 151 (12)                                   | <0.01 | 123 (12)                  | <0.01 | 102 (12) | <0.01 |

* P value was calculated by the Mann and Whitney U Test (10). The figures in column A at (1) was compared with the figures at (2), (3) and (4); Figures for each drug in column B were compared separately with the corresponding figures in Column A to determine the P value.

2) Effect of eserine, prostigmine, phosphamidon and mipafox on the release of acetylcholine from guineapig ileum in the PAM treated preparations

PAM was used at concentrations of 0.01 and 0.1 mM. The accumulation of acetylcholine in 15 minutes in PAM (0.01 mM) treated preparations, by eserine, prostigmine, phosphamidon and mipafox (each $1 \times 10^{-5}$ g/ml) was 581, 398, 189 and 123 ng/g respectively; With PAM, 0.1 mM, these values were 503, 341, 167 and 102 ng/g respectively (Table 1).
DISCUSSION

The release of acetylcholine from strips of small intestine, in situ or isolated, is well known (1-4, 11, 12). There have been reports of the effect of eserine and mipafox on the release of acetylcholine from guineapig ileum when these were used as anticholinesterases to protect the acetylcholine released from hydrolysis (1-4). The purpose of the present study was (i) to compare the effects of various inhibitors of cholinesterase—esserine, prostigmine, phosphamidon and mipafox, on the release of acetylcholine from isolated guineapig ileum under similar experimental conditions, (ii) to determine whether or not PAM which at certain concentrations reduces the release of acetylcholine from rat phrenic nerve diaphragm (8), has any effect on the release of acetylcholine from the guineapig ileum in the presence of anticholinesterases. Our results (Table 1) indicate that the accumulation of acetylcholine in the bath in the presence of eserine and prostigmine was 694 and 478 ng/g respectively. Eserine, besides the anticholinesterase activity, has other actions; it has a neurone stimulating action (13) and it induces acetylcholine release from the guinea pig tracheal chain preparation (5). It may produce an increased concentration of acetylcholine in the bath fluid by a mechanism different from its anticholinesterase activity. However, Ambache et al. (14) found that acetylcholinesterase activity of guineapig ileum was mainly localized in Auerbach’s plexus while that of butyrylcholinesterase mostly in the longitudinal smooth muscle layer. This distribution of acetylcholinesterase and butyrylcholinesterase may influence the hydrolysis of endogenously released acetylcholine which is mostly inactivated by acetylcholinesterase. Eserine inhibits both the butyrylcholinesterase and acetylcholinesterase (15) and gives complete protection to endogenously released acetylcholine; this causes greater accumulation of acetylcholine in the bath when eserine is used (Table 1). Prostigmine has similar but less anticholinesterase activity than eserine. It causes 478 ng/g accumulation of acetylcholine (Table 1) which is lower than the value obtained with eserine. Mipafox inhibits butyryl or pseudocholinesterase more effectively than acetylcholinesterase (16, 17) and hence does not give complete protection to endogenously released acetylcholine; consequently the value for acetylcholine release (151 ng/g) is lowest in the presence of mipafox (Table 1). Phosphamidon likewise causes 227 ng/g accumulation of acetylcholine which is greater than the value obtained with mipafox.

The release of acetylcholine, in the presence of PAM (0.01 mM), by eserine, prostigmine (both carbamates), phosphamidon and mipafox (both organophosphates) was 581, 398, 189 and 123 ng/g respectively while in its absence these values were 694, 478, 227 and 151 ng/g respectively (Table 1). PAM reduces the release of acetylcholine from guineapig ileum in the presence of carbamate as well as organophosphate compounds; with increased concentration of PAM (0.1 mM), the acetylcholine output is further reduced. It is difficult to say whether, or not, this effect of PAM is related to its cholinesterase reactivating effect. PAM reactivates the inhibited (phosphorylated) cholinesterase (6, 7) but has no such effect on the carboxamylated cholinesterase (18). PAM altered the release of acetyl-
choline from isolated rat diaphragm in the presence of prostigmine, a carbamate compound (8); Our results also indicate that PAM reduces the release of acetylcholine from guinea-pig ileum (Table 1) both in the presence of carbamate and organophosphate compounds. It therefore appears unlikely that the effect of PAM in reducing the release of acetylcholine would be related to its cholinesterase reactivating effect. It has been reported that certain substances which reduce the acetylcholine release from somatic nerve endings (19), have a similar effect on the release of acetylcholine from smooth muscle (20). As referred previously, PAM at certain concentrations, reduces the acetylcholine release from rat phrenic nerve diaphragm (8); Our results indicate that it also reduces the release of acetylcholine (Table 1) from smooth muscles of guinea-pig ileum in the presence of anticholinesterases.

SUMMARY

1. Effects of four anticholinesterases—eserine, prostigmine, phosphamidon and mipafox, on spontaneous release of acetylcholine from isolated guinea-pig ileum, under similar experimental conditions have been studied; Maximum value for acetylcholine release in the presence of eserine was obtained, followed by prostigmine, phosphamidon and mipafox in the order. The significance of the findings was discussed and an attempt was made to correlate it with their effect on acetylcholinesterase and butyrylcholinesterase in the smooth muscles of guinea-pig ileum.

2. It was found that PAM which at certain concentrations reduced the acetylcholine release from rat phrenic nerve diaphragm, had a similar effect on the release of acetylcholine from guinea-pig ileum in the presence of various anticholinesterases.

Acknowledgement: The authors are grateful to Dr. S.H. Zaidi, Director, Industrial Toxicology Research Centre, Lucknow, for his guidance in the above work, to Mr. Zubair Ahmad and Mr. P.A. George for their help in the statistical analysis of the results, and to Miss L. Sajnani for her technical assistance. The generous supply of PAM from Sumitomo Chemical Co. Ltd., Osaka, Japan and Phosphamidon from CIBA is gratefully acknowledged.

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