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THE FUNCTION OF TRUE AND PSEUDOCHOLINESTERASE IN THE MAMMALIAN ILEUM

DANA JAMIESON*

Pharmacology Department, University of Sydney

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Abstract—The responses of the mammalian intestine to nervously released and externally applied ACh was tested when either true cholinesterase (acetylcholinesterase) or pseudocholinesterase activity was inhibited. Nicotine was used to cause ACh release from nerve endings while ACh added to the bath represented ACh of non-nervous origin. The specific cholinesterase inhibitors used were BW 284C51 for true cholinesterase inhibition and *iso*OMPA for pseudocholinesterase inhibition. When true cholinesterase was inhibited in the rat, rabbit or guinea-pig, the responses to both nervously released ACh and ACh of non-nervous origin were potentiated; the former (nicotine responses) being much more increased than the latter. Pseudo-cholinesterase inhibition in the rat or guinea-pig potentiated responses to externally applied ACh only. However, the rabbit differed in that the action of both nervously released and added ACh were potentiated after pseudocholinesterase inhibition. Control experiments were done to eliminate possible direct effects of the inhibitors on the sensitivity of the preparation. It is concluded that true cholinesterase normally functions in the removal of both nervous and non-nervous ACh, while pseudocholinesterase, in the rat and guinea-pig, destroys only ACh of non-nervous origin.

SINCE the discovery of two distinct cholinesterase types[1] there has been controversy over the relative importance of the two enzymes in the role of destruction of acetylcholine (ACh) at various sites, and the reason for the existence of both enzymes. It has been postulated that pseudocholinesterase has no important function *in vivo*.[2–5] However, several reports indicate that pseudocholinesterase has a definite role in the destruction of ACh, particularly in the intestine.[6, 8] Studies of true (acetylcholinesterase) and pseudocholinesterase activity[9] have shown that pseudocholinesterase is the predominant cholinesterase in the intestine, and is present in large quantities. Pseudocholinesterase is distributed throughout the muscle layer and in both glial and ganglion cells, with particularly high concentrations in Auerbach's plexus[10–12] and its presence at such sites has been considered indicative of a function in the hydrolysis of nervously released ACh.

In the present work an attempt has been made to determine the relative importance of true and pseudocholinesterase in the hydrolysis of nervously released (neurogenic) acetylcholine, and acetylcholine of non-nervous origin in the intenstine. As non-neurogenic acetylcholine has been associated with spontaneous rhymicity,[13, 14] its destruction by pseudocholinesterase would provide an important physiological role for this enzyme,

Small doses of nicotine have been used to cause a release of ACh from the nerve endings, thus representing neurogenic ACh, while ACh added to the bathing fluid was taken to represent ACh of non-nervous origin.

* Present address: Radiobiology Research Unit, Cancer Institute Board, 278 William Street, Melbourne, C.1, Australia.

Intestinal true and pseudocholinesterase were differentially inactivated by specific inhibitors and the effect on the responses to nicotine and to ACh was observed. With the specific antagonist of true cholinesterase BW 284C51 and the specific anti-pseudocholinesterase *iso*OMPA it is possible to inhibit almost completely one cholinesterase whilst leaving the other virtually unaffected, and to observe resultant changes in the response of the tissue. If depression of one of the cholinesterases in the tissue causes an augmented response to nicotine of ACh then that enzyme presumably functions normally in the removal of nervous or non-nervous ACh respectively at that site.

METHODS

All animals were killed by a blow on the head or back of the neck. A 3–4 cm portion of ileum was suspended in Tyrode's solution aerated with 95% O_2 5% CO_2 (rat and rabbit ileum) or 100% O_2 (guinea-pig ileum). The bicarbonate concentration was 1·1 g/l. in the former and 1·0 g/l. in the latter case. Contractions of the longitudinal muscle were recorded with a frontal writing lever. The temperature of the bathing fluid was maintained at 37 $\pm$ 0·2°. Bathing fluid was changed by flooding from below to avoid exposure of the tissue to air.

When uniform responses were obtained on the ileum, doses of the stimulant drugs were adjusted until they were approximately equiactive. At least 2 dose levels of each stimulant drug were applied to the tissue to establish approximately the dose-response relationship and after treatment with the cholinesterase inhibitor these same doses were repeated. The approximate degree of potentiation could then be estimated graphically. For BW 284C51 which is a reversible inhibitor the doses were again repeated after the inhibitor was removed to test recovery.

Cholinesterase inhibitors, and hexamethonium, were added to the reservoir. Rapid removal of inhibitors was effected by using two reservoirs of bathing fluid connected to the organ bath via a two-way tap.

Doses of stimulant drugs were applied each 90 sec to guinea-pig and rat ileum and each 2 min to rabbit ileum, and left in contact with the tissue for 30 sec. The concentration of inhibitor used in each experiment was as low as practicable and was determined by several factors. Low concentrations minimise possible direct actions on cholinergic receptors; in addition the specificity of inhibition is high. Thus doses of anticholinesterases were chosen which would give a readily measurable degree of potentiation, and these doses varied with the species and in individual experiments.

Drugs used in this work were acetylcholine chloride (ACh); nicotine acid tartrate; acetyl-β-methylcholine bromide; carbaminoylcholine chloride; (Carbachol), hexamethonium iodide; atropine sulphate; 1,5-di-(*p*-n-allyl-n-methyl aminophenyl) pentan-3-one dibromide (BW 284C51, kindly supplied by Dr. F. C. Copp); nn'diisopropyl phosphorodiamidic anhydride (*iso*OMPA, kindly supplied by the Australian Chemical Defence Laboratories). All doses of drugs refer to the corresponding salts or complexes of these substances.

RESULTS

Inhibition of true cholinesterase by BW 284C51

The anticholinesterase activity of BW 284C51, a selective inhibitor of true cholinesterase, was examined by Fulton and Mogey[15] and Austin and Berry[16] in several

species including the guinea-pig. These results were extended to the rat and rabbit[12] and the compound has been used in the isolated rabbit ileum.[17] On addition of BW 284C51 to the perfusion fluid an initial rise in tone occurred followed by relaxation to the original baseline after some minutes. Potentiation of the responses to stimulant drugs occurred within several minutes of adding the inhibitor. However, recovery was slow; for the rat and rabbit ileum the initial sensitivity was not restored until up to 30 min after BW 284C51 was removed from contact with the tissue. In the guinea-pig ileum where very low concentrations of the inhibitor were used recovery was complete within 5 to 10 min following removal of the anticholinesterase.

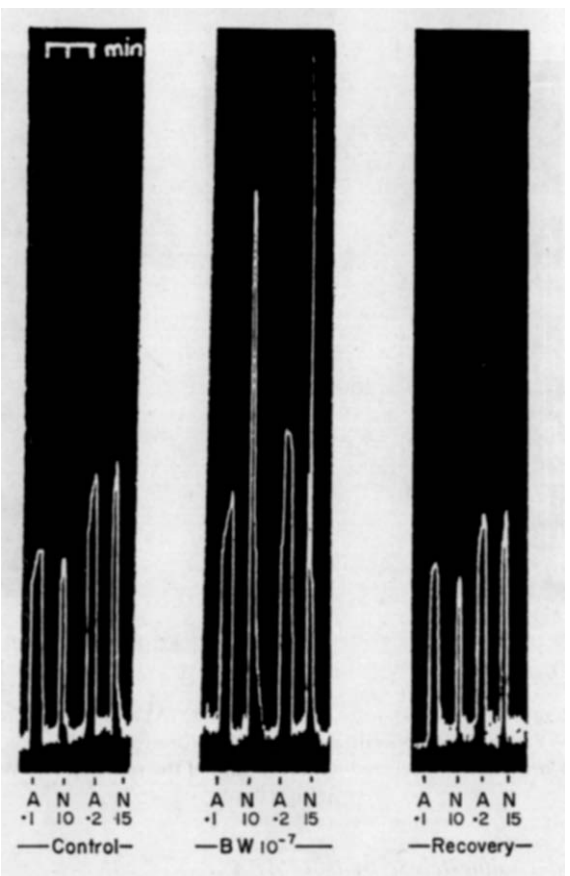

FIG. 1. Rat ileum. In the presence of BW 284C51 (BW) (centre panel) in a final concentration of 10^{-7} the responses to ACh (A) were potentiated, and those to nicotine (N) more greatly potentiated. Recovery (final panel) occurred 30 min after removal of the anticholinesterase. Doses of motor drugs μg/10 ml.

Rat ileum. In six of six experiments both ACh and nicotine responses were potentiated by BW 284C51 ($1–5 \times 10^{-7}$ g/ml) (Fig. 1). The potentiation of nicotine was five to ten times greater than that of ACh as estimated from dose-response curves for these substances.

Guinea-pig ileum. As in the rat ileum both ACh and nicotine were potentiated by BW 284C51 in all six trials. However, very low doses of this inhibitor were used ($2{\cdot}5 \times 10^{-9}$ to 10^{-8}) on guinea-pig preparations, as larger doses caused a prolonged contraction and subsequent loss of sensitivity. Again, contractions produced by nicotine were increased more than those produced by acetylcholine (Fig. 2).

Rabbit ileum. In all eight experiments both ACh and nicotine were potentiated by BW 284C51 ($1{\cdot}5 \times 10^{-7}$). Response heights in the rabbit ileum were measured from the mid-point of the pendulum movements before and after addition of the stimulant drug as described by Ambache and Lessin.[18] Nicotine responses were always augmented to a greater extent than ACh responses.

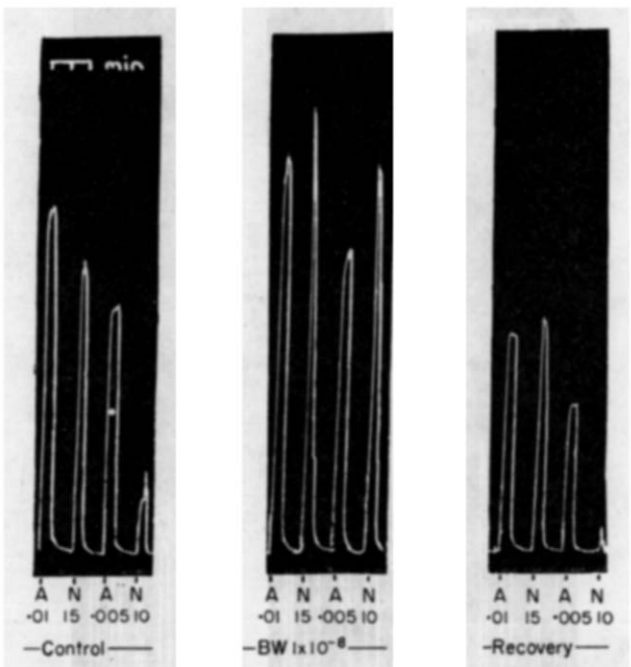

FIG. 2. Guinea-pig ileum. Control responses to acetylcholine (A) and to nicotine (N) were potentiated by BW 284C51 (BW) in a final concentration of 10^{-8} (centre panel) with nicotine potentiation exceeding that of ACh. Recovery occurred within 10 min of the removal of BW. Doses are expressed in μg/10 ml.

Inhibition of pseudocholinesterase by isoOMPA

*Iso*OMPA in a concentration of approximately $1{\cdot}5 \times 10^{-5}$ is an irreversible inhibitor of pseudocholinesterase, causing 95 per cent inhibition of pseudocholinesterase with not more than 5–10 per cent inhibition of true cholinesterase in all species tested.[16] The onset of action is relatively slow and progressive.[19–21] Thus after control responses to the stimulant drugs were obtained *iso*OMPA was kept in contact with the tissue for a fixed period of 30 min in each experiment, then removed from contact with the tissue prior to the re-addition of stimulant drugs.

Rat ileum. Concentrations of *iso*OMPA necessary to produce potentiation varied from 5×10^{-6} to 2×10^{-5}. ACh contractions were increased by this inhibitor in all six

trials. The responses to nicotine were not potentiated in any experiment, but slightly depressed in four cases.

Guinea-pig ileum, *Iso*OMPA (5×10^{-6}–2×10^{-5}) consistently augmented the responses to ACh. Nicotine responses were only slightly increased in three of six experiments. (Fig. 3).

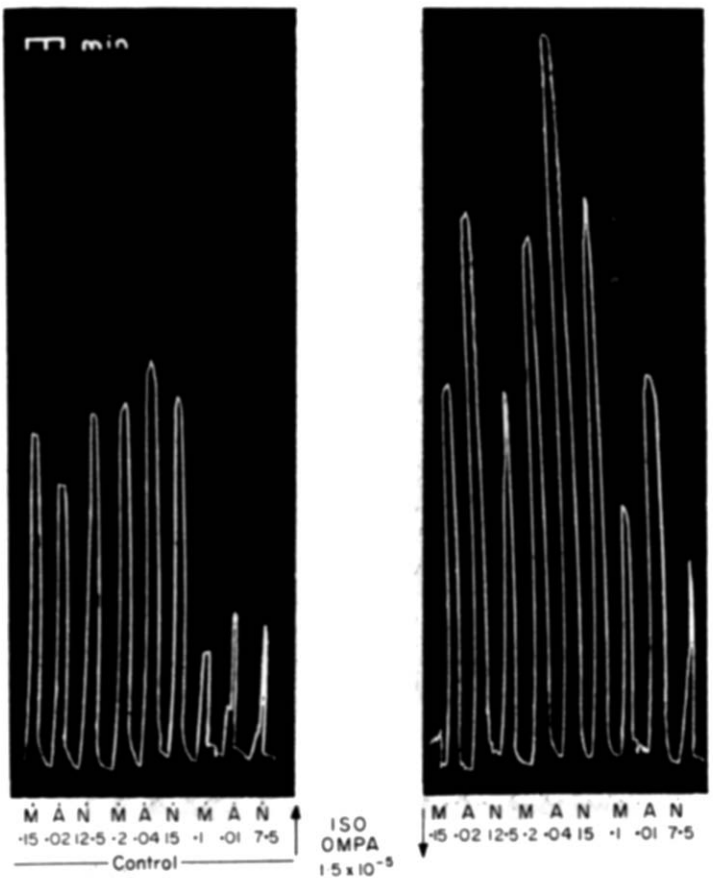

FIG. 3. Guinea-pig ileum. ACh (A) responses were potentiated after 30 min treatment with *iso*OMPA to a considerably greater degree than the responses to the control drug acetyl-β-methylcholine (M). Nicotine (N) responses are nearly unchanged compared to responses of the control drug. Doses of stimulants in μg/10 ml. Arrows indicate addition and removal of *iso*OMPA in this and subsequent Figs.

Rabbit ileum. In all eight experiments treatment with *iso*OMPA (5×10^{-6}–2×10^{-5}) caused potentiation of the responses to ACh. Nicotine responses were also potentiated in this tissue to a considerably greater degree than those to ACh (Fig. 4).

Control experiments

Certain cholinesterase inhibitors produce cholinomimetic responses in doses which are estimated to cause relatively little cholinesterase inhibition. Consequently their primary effects have been ascribed chiefly to a direct action at cholinoreceptive

sites.[22, 23] Other inhibitors produce anticholinergic responses: an atropine-like action has been described by[24] for several anticholinesterases, including BW 284C51. This latter observation has been confirmed by Ambache and Lessin.[18] The possibility existed that the anticholinesterases used in this work had a direct sensitising or depressing action on the ileum, which would interfere with the potentiation of response

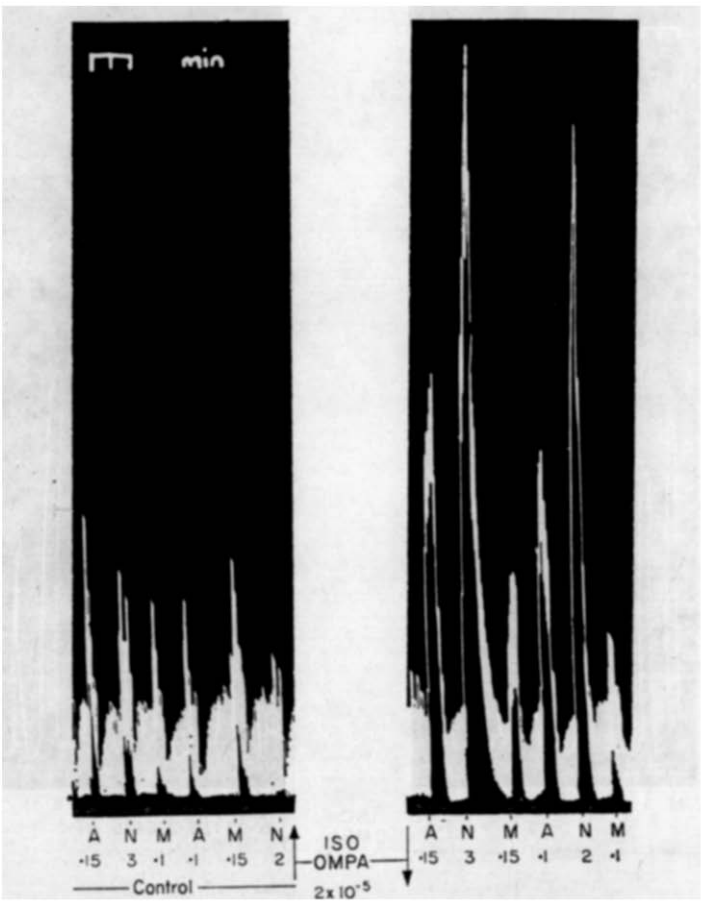

FIG. 4. Rabbit ileum. Nicotine (N), as well as ACh (A), was potentiated by *iso*OMPA (2×10^{-5}) left in contact with the tissue for 30 min. Although in this species nicotine responses were increased more than those to ACh, the responses to the control drug, acetyl-β-methylcholine (M) were slightly depressed.

due solely to anticholinesterase activity. Thus it was necessary to study the interaction of these inhibitors with a directly acting (musculotropic) stimulant drug, which would itself be refractory to hydrolysis by cholinesterase. Some difficulty was experienced in finding compounds suitable for this purpose. Inorganic cations and histamine, formerly thought to be purely muscle stimulants, have been shown to excite nervous elements.[25–30] Angiotensin was found to act indirectly in the intestine,[31–34] while substance P is sometimes potentiated by BW 284C51.[17]

The muscarinic compound, acetyl-β-methylcholine provided a suitable control in experiments where pseudocholinesterase was inhibited as it is devoid of neurotropic action and not hydrolysed by pseudocholinesterase. No such parallel substance was available for experiments where true cholinesterase was inhibited. Thus the partly muscarinic drug carbachol was used as a control, in the presence of hexamethonium to abolish the ganglionic action of the drug. This leaves a muscarinic component which is not hydrolysed by cholinesterase and should be unaffected by anticholinesterase action. Concentrations of hexamethonium sufficient to abolish stimulant test doses of nicotine were used. These were 5×10^{-5}–10^{-4} in the rat and guinea-pig preparations

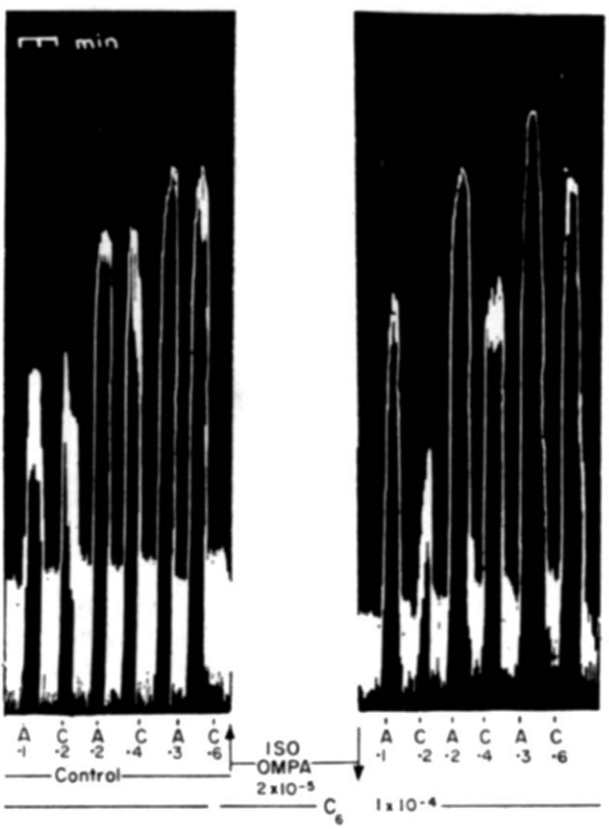

FIG. 5. Rabbit ileum. Approximately equivalent control responses to ACh (A) and carbachol (C) were obtained in the presence of hexamethonium (C_6). *Iso*OMPA applied to the tissue for 30 min potentiated ACh, yet slightly depressed the sensitivity of the intestine as shown by the carbachol responses. Doses in μg/10 ml.

and up to 2×10^{-4} in the rabbit preparations. Dose-response curves were obtained for carbachol in the presence of hexamethonium before and after anticholinesterase treatment. Acetylcholine was given in the same experiment to observe whether the potentiating effect of the anticholinesterases on acetylcholine responses were altered by the presence of the ganglion blocking agent.

Control experiments with iso*OMPA*

Interaction of acetyl-β-methylcholine with iso*OMPA.* Control responses to acetyl-β-methylcholine were obtained together with those to ACh and nicotine in the experiments with *iso*OMPA (Figs. 3 and 4). In the rat this anti-pseudocholinesterase very slightly depressed the acetyl-β-methylcholine responses, together with a similar depression in the nicotine contractions; while in the guinea-pig parallel slight increases in acetyl-β-methylcholine and nicotine responses occurred. *Iso*OMPA depressed acetyl-β-methylcholine responses to a moderate degree in rabbit ileal preparations.

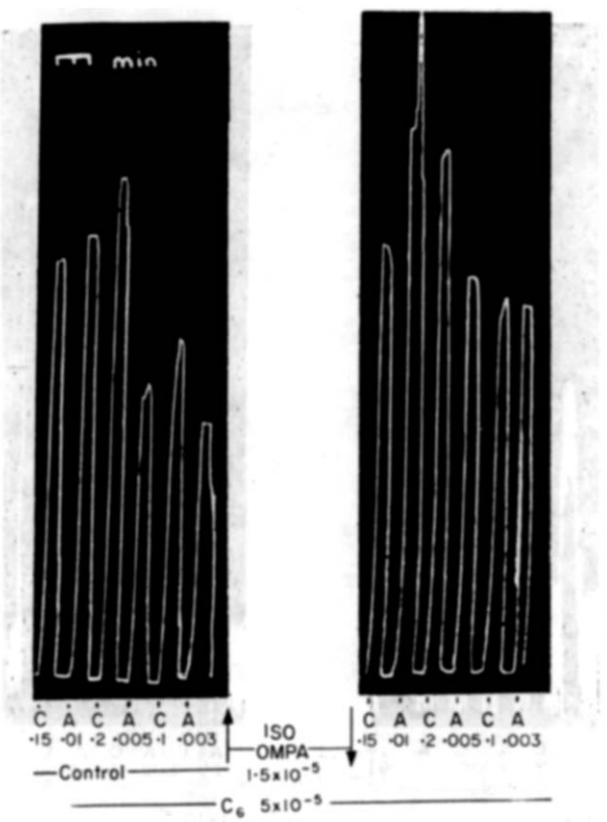

FIG. 6. Guinea-pig ileum. This Fig. illustrates the effect of 30 min contact with *iso*OMPA ($1{\cdot}5 \times 10^{-5}$) on the sensitivity of the guinea-pig ileum to ACh (A) and carbachol (C) in the presence of hexamethonium (C_6). The responses to carbachol were almost unchanged by *iso*OMPA treatment while acetylcholine responses increased. Doses of stimulant drugs in μg/10 ml.

Interaction of cabachol and ACh with iso*OMPA in the presence of hexamethonium*

The results using carbachol as a control confirmed those with acetyl-β-methylcholine, i.e. *iso*OMPA in the same concentrations as used previously, had a slight direct action on most of the ileal preparations. This action was depressant in the rat and rabbit ileum (Fig. 5). There was either no effect or a slight stimulant action on the guinea-pig ileum (Fig. 6). The presence of hexamethonium had no appreciable effect on the

potentiation of ACh by *iso*OMPA. Although *iso*OMPA sometimes exerts a slight direct ACh-like action on the guinea-pig ileum, the augmentation of ACh responses was always considerably greater than the corresponding increase in carbachol contractions (Fig. 6). At least four experiments were done for each series.

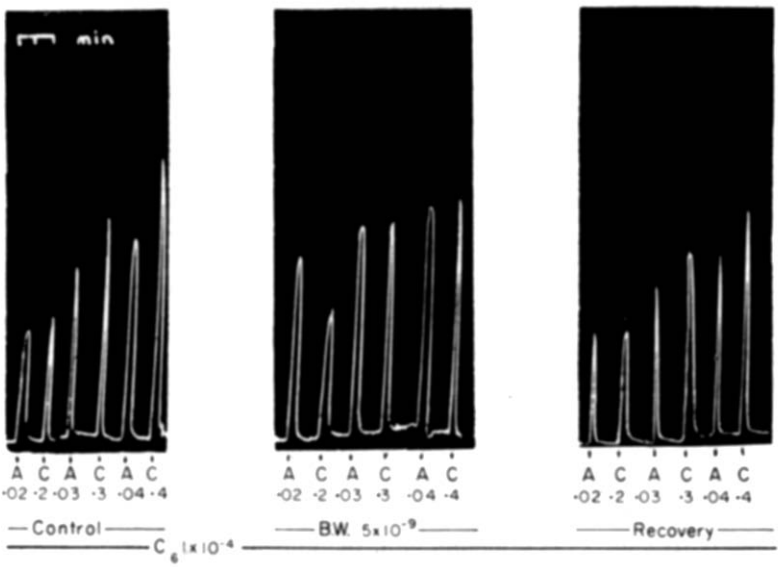

FIG. 7. Guinea-pig ileum. Control responses to ACh (A) and carbachol (C) were obtained in the presence of hexamethonium (C_6). In the presence of BW 284C51 (Centre panel) the responses to ACh were potentiated, without a change in the responses to carbachol. Recovery within 10 min is shown in the final panel.

Control experiments with BW 284*C*51

Interaction of carbachol and ACh with BW 284*C*51 *in the presence of hexamethonium* BW 284C51 did not affect the responses to carbachol in the guinea-pig ileum (Fig. 7) and had a very slight atropine-like action on the rat ileum and a somewhat greater atropine-like action on the rabbit ileum. The presence of hexamethonium did not affect the degree of potentiation of ACh responses by BW 284C51.

DISCUSSION

Inhibition of true cholinesterase in the three species tested potentiated neurogenic ACh (nicotine responses) and also added ACh, the former being potentiated to a considerably greater degree. Thus it appears that cholinesterase normally hydrolyses ACh from both sources, but is more effective in hydrolysing nervously released ACh.

This effect could be explained by the location of the enzyme. High densities of true cholinesterase are concentrated at the nerve endings and it is also known to occur throughout the muscle fibres.[11, 12] As it may be more diffusely scattered throughout the muscle than at the neuro-effector junction its action against external ACh would not be as rapid as against nervously released ACh and thus inhibition of true cholinesterase would produce a more pronounced effect on neurogenic ACh. An alternative explanation is based on the theory advanced by Langley[35] and discussed more fully by Ambache;[36] the possibility that interneurones exist between the pre- and

post-ganglionic fibres of motor nerves in the intestine. If inhibition of cholinesterase causes potentiation of ACh at the ganglion as well as at the neuroeffector junction, the final response to nicotine would be greatly increased, as the effect of ACh would be rendered progressively greater at each intermediate synapse.

Pseudocholinesterase does not appear to function in the destruction of nervously released ACh in the rat and guinea-pig ileum, for nicotine responses are not potentiated in these species by pseudocholinesterase inhibition. However, in the rabbit the responses to nervously released ACh were greatly potentiated. The control drug acetyl-β-methylcholine was not affected by the pseudocholinesterase inhibitor *iso*-OMPA, showing that the doses used were selective for the inhibition of pseudocholinesterase, and no direct sensitisation was produced. This was further confirmed using carbachol as a control. It is therefore concluded that pseudocholinesterase functions in the removal of neurogenic ACh in the rabbit intestine.

In all the species tested inhibition of either true or pseudocholinesterase increased the responses to externally applied ACh, thus it appears that both enzymes participate in the hydrolysis of non-nervous ACh.

That the potentiation of ACh occurs at the neuroeffector junction, and is not attributable to a potentiation of the possible slight nicotinic effect of ACh added to the bath, is shown by the interaction of ACh, hexamethonium and the anticholinesterases. In the presence of the ganglion-blocking agent hexamethonium the responses to ACh were still increased. As shown by the use of the control substances acetyl-β-methylcholine, and carbachol + hexamethonium, the anticholinesterases in the concentrations used in this work show relatively slight actions, apart from cholinesterase inhibition, on intestinal smooth muscle. Such controls were essential in view of the evidence of direct actions of anti-cholinesterases particularly at the neuromuscular junction.[15, 37–40] Low concentrations of the inhibitors were used throughout these experiments to minimise side actions. Some of the effects attributed to anticholinesterase action in earlier uncontrolled experiments may have been due, at least in part, to direct actions of the inhibitors used and may account for the controversial reports in the literature.

No attempt has been made to quantitatively correlate changes in responses of the isolated tissue to the degree of cholinesterase inhibition, as it has been pointed out that the same dose of anticholinesterase may produce different degrees of inhibition in whole tissue and in homogenised tissue.[41, 42]

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