Role of butyrylcholinesterase in canine tracheal smooth muscle function*

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The role of butyrylcholinesterase (BuChE) and acetylcholinesterase (AChE) in regulating acetylcholine (ACh) lifetime was investigated by use of selective cholinesterase (ChE) inhibitors. Addition of 1 μM tetraisopropylpyrophosphoramide (iso-OMPA) led to a 98% inhibition of BuChE activity with little or no effect on AChE activity This inhibition wa accompanied by a 26% increase in the amplitude and a 43% prolongation in the half-relaxation time of contractions elicited by electric field stimulation (EFS) Coapplication of BW 284C51 (a selective AChE inhibitor) and 1 μM iso-OMPA resulted in increases of 2-fold in the amplitude and 10-fold in the half-relaxation time of EFS-induced contractions. These alterations were accompanied by small but sustained baseline contractures that were antagonized completely by incubation with exogenous BuChE (2.5 U/ml). The results suggest that BuChE serves to coregulate the lifetime of ACh in canine tracheal smooth muscle.

Pseudocholinesterase; Acetylcholinesterase, Tetraisopropylpyrophosphoramide, BW 284C51

1. INTRODUCTION

At all cholinergic synapses thus far investigated, acetylcholine (ACh) action is terminated by rapid hydrolysis of excess transmitter. This hydrolysis is normally mediated by the enzyme acetylcholinesterase (AChE; EC 3.1.1.7; acetylcholine hydrolase), which rapidly degrades ACh into choline and acetate [1]. Another ACh hydrolyzing enzyme, butyrylcholinesterase (BuChE; EC 3.1.1.8; acylcholine acylhydrolase; pseudocholinesterase) is found primarily in plasma but is also present in appreciable quantities in cardiac and smooth muscle fibers and autonomic ganglia [2–5]. Unlike AChE, BuChE has generally been considered to have no defined physiological function [5,6]. This stems, in part, from the low activity of BuChE in most cholinergic synapses such as those in skeletal muscle and the CNS [7].

In canine tracheal smooth muscle (TSM), cholinesterase (ChE) inhibitors produce an increased sensitivity to exogenous ACh, an enhanced contraction in response to electric field stimulation (EFS) and a sustained increase in baseline tension [8,9]. It is not clear whether these alterations result from inhibition of AChE, BuChE or both since most ChE inhibitors show little selectivity between the two enzymes. A contribution by AChE is likely, based on its well known role in hydrolyzing ACh at neuromuscular and ganglionic synapses [10,11]. A contribution by BuChE is also possible, since in canine TSM, this enzyme comprises the majority of ChE active sites [8]. However, to be functional, BuChE must have a sufficiently high substrate affinity and catalytic activity to eliminate excess ACh and prevent transmitter accumulation.

To test the possibility that BuChE has a functional role in canine TSM, we examined the actions of the selective inhibitor of BuChE (tetraisopropylpyrophosphoramide, iso-OMPA) and of AChE (1,5-(bis(*N*-allyl-*N*,*N*-dimethyl-4-ammoniumphenyl)-pentane-3-one dibromide, BW 284C51), respectively, on spontaneous and EFS-elicited muscle tensions. Inhibition of either BuChE or AChE resulted in slight to moderate increases in EFS-induced contractions whereas combined inhibition of both enzymes led to a marked potentiation of such contractions as well as the production of sustained baseline tension. The results indicate that BuChE contributes to ACh hydrolysis in canine TSM; this effect is most pronounced when AChE is also inhibited.

2. MATERIALS AND METHODS

2.1. *Muscle tension*

Tracheae were removed from mongrel dogs under pentobarbital anesthesia (30 mg/kg, i.v.), freed of loose connective tissue and mucosa and sectioned into 2 × 10 mm rectangular strips. Isometric tensions were recorded at 37°C in 10 ml chambers containing oxygenated (95% O_2, 5% CO_2) Tyrode's solution of the following composition (mM): NaCl, 137; KCl, 2.7; $NaHCO_3$, 11.9; NaH_2PO_4, 0.3; $MgCl_2$, 0.5; $CaCl_2$, 1.8; glucose, 5.6; pH 7.4. Tensions were recorded

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with Grass FT 0.03 force displacement transducers and displayed on a Gould chart recorder and Honeywell FM tape recorder. TSM strips were equilibrated for 1 h prior to recording at a resting tension of 4 g. Contractions were elicited by electrical pulses applied between pairs of platinum electrodes via Grass S-88 stimulators. The pulses were 50 V and 1 ms delivered in trains of 10 Hz for 20 s.

2.2. *ChE activity*

Unless stated otherwise, a radiometric assay was used to measure ChE activity [12]. The experiments were performed on strips to limit the responses to surface enzymes [13]. Total activity was determined by use of [^{14}C]ACh, whereas BuChE and AChE activities were evaluated by use of [^{14}C]butyrylcholine (BuCh) and [^{14}C]acetyl-β-methylcholine (MeCh), respectively. Selective inhibition of AChE and BuChE was achieved by addition of BW 284C51 and iso-OMPA, as appropriate. The reaction was terminated by addition of Amberlite CG-120 suspended in 1,4-dioxane to remove unhydrolyzed substrate. The samples were centrifuged briefly to sediment the Amberlite. The radioactivity in the supernatant was counted in a Beckman scintillation spectrometer.

2.3. *Kinetic constants*

To obtain kinetic data, ChE activity was measured by the procedure of Ellman et al. [14]. Total activity was assessed by incubating muscle homogenates at 37°C and pH 8.0 with acetylthiocholine at concentrations ranging from 10 μM to 30 mM in a Titertek Multiscan Model MCC plate reader (Flow Labs, Mclean, VA). The contributions of AChE and BuChE were evaluated by preincubating the homogenates for 30 min with 1 μM iso-OMPA or 1 μM BW 284C51, as appropriate. The parameters K_m and V_{max} were estimated from double-reciprocal plots of substrate-velocity data in the low substrate concentration range (10 μM to 1 mM).

2.4. *Materials*

BW 284C51, iso-OMPA, acetylthiocholine chloride and butyrylthiocholine chloride and BuChE type VII were obtained from the Sigma Chemical Co., St. Louis, MO. Radiolabeled substrates were purchased from New England Nuclear Research Products (Dupont), Boston, MA. Soman was synthesized by the Chemical Research, Development and Engineering Center, Aberdeen Proving Ground, MD.

3. RESULTS

3.1. *Differential and combined inhibition of ChE*

The effects of BW 284C51 and iso-OMPA on EFS-induced contractions are shown in Fig. 1, and the corresponding alterations in ChE activities are displayed in Fig. 2. In the presence of 1 μM BW 284C51, the EFS-evoked contractions increased by 76 ± 6.3% in amplitude and by 129 ± 8.1% in duration (Fig. 1). Under these conditions, AChE activity was reduced to 22% of control, as indicated by the impaired hydrolysis of MeCh, while BuChE activity was not significantly altered (Fig. 2).

Addition of 1 μM iso-OMPA led to increases of 26 ± 2.3% and 43 ± 5.9%, respectively, in the amplitude and half-relaxation time (HRT) of the EFS-induced contractions (Fig. 1). At 1 μM, iso-OMPA produced a selective and nearly total abolition of BuChE activity (Fig. 2). The alterations induced by iso-OMPA were irreversible, as indicated by the absence of recovery even after a 1 h wash in control Tyrode's solution (Fig. 1).

Reapplication of BW 284C51 to preparations pretreated with iso-OMPA or coapplication of BW 284C51 with iso-OMPA caused a further increase in the contraction amplitude and a striking prolongation in its decay (Fig. 1). The effect of the combined ChE inhibitors on the HRT was considerably greater than that observed with either inhibitor alone or than that expected from their linear addition. Since, at the concentrations used, there is little overlap in the specificities of BW 284C51 and iso-OMPA on enzyme activity (Fig. 2), the potentiation must result from the simultaneous inhibition of both enzymes.

3.2. *Antagonism by exogenous BuChE*

To substantiate the role of BuChE in coregulating ACh lifetimes in canine TSM, we examined the effects of exogenous BuChE on EFS-induced tensions and sustained muscle contractures. The results of a representative experiment are shown in Fig. 3. A marked potentiation was observed in the EFS-induced contraction by co-applying 1 μM BW 284C51 with 1 μM iso-OMPA for 30 min (Fig. 3A). These alterations were accompanied by a 4.2 g increase in the baseline tension

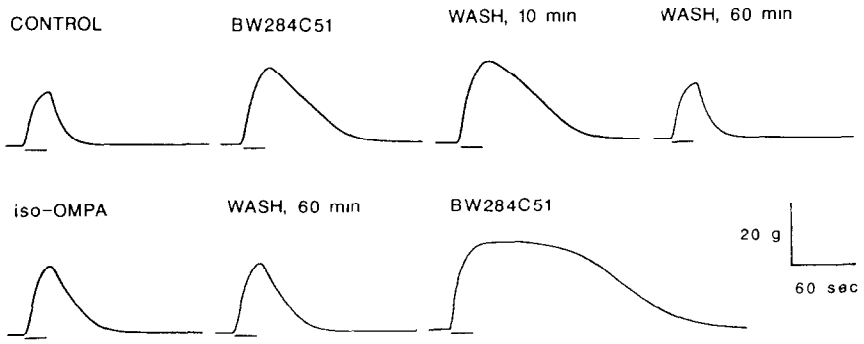

Fig. 1. Traces illustrating the differential and combined inhibition of AChE and BuChE on EFS-induced contractions from canine TSM. The data were obtained from one muscle strip exposed to the inhibitors BW 284C51 (1 μM) and iso-OMPA (1 μM) in the sequence shown. An equilibration period of ≥30 min preceded the recordings. The initial 10 min of BW 284C51 washout was accompanied by an increase in the amplitude and HRT of the EFS-induced contraction followed by a return to the control configuration with continued wash. The transient potentiation results from a rapidly reversible antimuscarinic action of BW 284C51. The last trace was obtained following reapplication of BW 284C51 after irreversible inhibition of BuChE by iso-OMPA. This response is superimposed on a 3.4 g contracture (not shown).

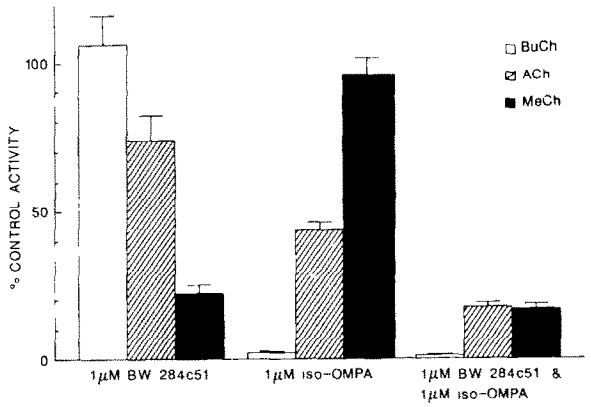

Fig. 2. Histograms showing the effects of BW 284C51 and iso-OMPA on surface ChE activity in canine TSM. The data were obtained by incubating 9–12 muscle strips in BW 284C51, iso-OMPA or both for 30 min and assaying for BuChE, AChE and total ChE activities using the substrates BuCh, MeCh and ACh, respectively. Bars denote the SE.

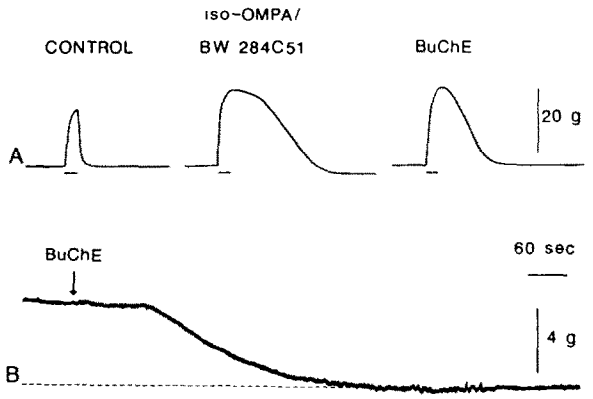

Fig. 3. Effect of exogenous BuChE on EFS-induced contractions and basal tone. (A) A 30 min exposure to 1 μM iso-OMPA and 1 μM BW 284C51 caused a marked potentiation in the amplitude and HRT of EFS-induced contractions. The effect on HRT was partially antagonized following a 40 min incubation with BuChE (Sigma type VII, 2.5 U/ml). Train durations were for 20 s as indicated by the horizontal bars. (B) BuChE (2.5 U/ml) produced complete relaxation of the 4.2 g contraction resulting from combined addition of BW 284C51 and iso-OMPA. Baseline is indicated by the interrupted line.

(Fig. 3B). Excess iso-OMPA was removed by washout with a solution containing only the reversible BW 284C51, and a commercial preparation of BuChE (Sigma type VII) was then added to the bathing medium. As is clear, the duration of the EFS-induced contraction was markedly reduced following a 40 min incubation with BuChE (2.5 U/ml). Exogenous BuChE also caused complete relaxation of the contracture. Both effects persisted for up to 3 h but could be reversed by washout with enzyme-free solution or readmission of iso-OMPA (unpublished observations). These results are of interest, since they reveal the ability of diffusely applied BuChE to hydrolyze ACh released both under resting conditions as well as during stimulation. The former is considered to represent nonquantal release [15].

3.3. *Determination of kinetic constants*

Since BuChE apparently contributes to transmitter hydrolysis in canine TSM but has not been reported to have this action in most preparations [7], it was of interest to determine whether BuChE from canine TSM possesses unique catalytic properties, i.e. to test whether its activity in airway smooth muscle is unusually high.

Fig. 4 shows the relationship between substrate concentration and hydrolytic activity for AChE and BuChE as determined by the colorimetric method of Ellman et al. [14]. AChE activity increased initially with increases in acetylthiocholine concentration, became maximal at approximately 1 mM and decreased thereafter (Fig. 4A). The decrease in activity is due to substrate inhibition, a characteristic property of AChE [16]. From double reciprocal plots in the low substrate concentration range (10 μM to 1 mM), values for V_{max} and K_m were estimated to be 2.8 ± 0.23 μmol/min/g

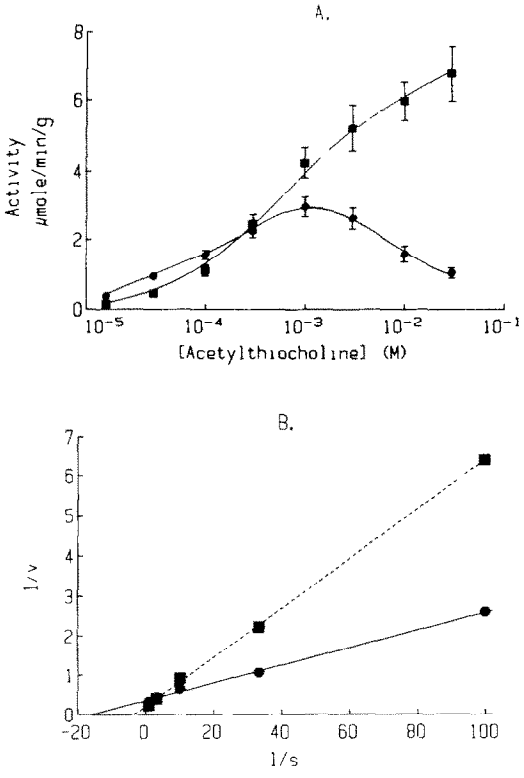

Fig. 4. (A) Substrate-velocity plots for hydrolysis of acetylthiocholine in canine TSM homogenates. AChE (circles) and BuChE (squares) activities were obtained by use of iso-OMPA and BW 284C51, respectively. The points represent the mean ± SE (n = 6) of initial velocities determined by the colorimetric method of Ellman et al. [14]. (B) Double reciprocal plots of the means of the data in (A) over the substrate concentration range 10 μM to 1 mM. The units for the ordinate and abscissa are $(\mu mol/min/g)^{-1}$ and (mM^{-1}), respectively.

tissue and 87 ± 11 μM, respectively (n = 6) (Fig. 4B). The latter is in accord with values for AChE obtained from brain, skeletal muscle and erythrocytes [7].

BuChE was less active than AChE at acetylthiocholine concentrations ≤ 100 μM but showed greater activity at higher substrate concentrations. This enzyme also deviated from simple Michaelis-Menten kinetics. However, it was activated rather than inhibited by high substrate concentrations [17]. From double-reciprocal plots, K_m for BuChE was calculated to be 634 ± 81 μM (n = 6). This value is similar to those reported for BuChE from plasma [5], autonomic ganglia [3] and intestinal smooth muscle [13]. V_{max} for BuChE could not be determined accurately, since the substrate-velocity relationship did not saturate even with 30 mM acetylthiocholine (Fig. 4A).

4. DISCUSSION

The role of AChE in regulating cholinergic synapses is well established, but there is no general agreement regarding the role of BuChE. An impression that BuChE is not involved in cholinergic transmission originated from findings that inhibition of this enzyme in vivo produces no obvious toxicity [18]. This impression has been reinforced by findings that humans with relatively inactive genetic variants of BuChE do not exhibit signs of impaired cholinergic function [19]. However, as early as 1950, Koelle et al. [2] demonstrated that selective inhibition of BuChE leads to enhanced contractility in isolated ileal strips. The apparent discrepancy between this and the above results can be reconciled by noting that in vertebrates there is little BuChE activity in critical tissues such as brain and skeletal muscle; even where BuChE is substantial, it is always accompanied by AChE [19].

The results of the present investigation provide evidence for a functional role of BuChE in canine TSM. This conclusion is based on findings that selective inhibition of BuChE leads to significant increases in the amplitude and HRT of EFS-evoked contractions. Although functional, the efficacy of BuChE was considerably lower than that of AChE. This was indicated by findings that 1 μM BW 284C51, which inhibited 78% of AChE, produced more pronounced alterations of EFS-induced contractions than did 1 μM iso-OMPA, which inhibited 98% of BuChE (Figs 1 and 2). The relatively greater effect of AChE blockade may be explained by its higher rate of ACh hydrolysis at concentrations below 1 mM (Fig. 4). The differences in the catalytic properties of the two enzymes are reflected in the 7.3-fold lower K_m (higher affinity) for AChE relative to BuChE when acetylthiocholine was used as substrate.

It is appropriate at this time to ask what role BuChE plays in the normal physiology of canine TSM. Although shown to coregulate ACh lifetime in the present study, the use of BuChE for this purpose may appear to be inefficient since a much larger number of BuChE than AChE molecules are required to produce equivalent catalysis. However, since BuChE is activated and AChE is inhibited with high substrate concentrations (Fig. 4A), BuChE becomes the dominant enzyme when substrate concentrations exceed 1 mM. Thus, the primary role of BuChE may be to hydrolyze transmitter under conditions of high release and therefore to protect the TSM from excess ACh levels.

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