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pH-dependent hydrolysis of acetylcholine: Consequences for non-neuronal acetylcholine

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ABSTRACT

Acetylcholine is inactivated by acetylcholinesterase and butyrylcholinesterase and thereby its cellular signalling is stopped. One distinguishing difference between the neuronal and non-neuronal cholinergic system is the high expression level of the esterase activity within the former and a considerably lower level within the latter system. Thus, any situation which limits the activity of both esterases will affect the non-neuronal cholinergic system to a much greater extent than the neuronal one. Both esterases are pH-dependent with an optimum at pH above 7, whereas at pH values below 6 particularly the specific acetylcholinesterase is more or less inactive. Thus, acetylcholine is prevented from hydrolysis at such low pH values. The pH of the surface of the human skin is around 5 and therefore non-neuronal acetylcholine released from keratinocytes can be detected in a non-invasive manner. Several clinical conditions like metabolic acidosis, inflammation, fracture-related haematomas, cardiac ischemia and malignant tumours are associated with local or systemic pH values below 7. Thus, the present article describes some consequences of an impaired inactivation of extracellular non-neuronal acetylcholine.

1. Introduction

Acetylcholine and additional components of the non-neuronal cholinergic system (choline uptake, choline acetyltransferase, nicotinic and muscarinic receptors, esterases) have been demonstrated in epithelial, endothelial, mesothelial, immune cells as well as smooth muscle fibres [1–5]. Non-neuronal acetylcholine acts as a local cell molecule via autocrine and paracrine mechanisms to control basic cell functions such as proliferation, differentiation, maturation, migration, secretion, organization of the cytoskeleton and cell–cell contact [1–5].

Important differences exist between the organization of the non-neuronal and neuronal cholinergic systems, particularly with respect to the inactivation of extracellular acetylcholine. Release of acetylcholine from central or peripheral cholinergic neurones (for example myenteric plexus) or from motor neurones can be measured only when the cholinesterase is blocked, i.e. in the presence of inhibitors of the enzyme. In contrast, the release of non-neuronal acetylcholine from the placenta, murine stem cells, the human skin, human pancreatic alpha cells or small cell lung tumour cell lines can be detected in the absence of cholinesterase inhibitors [5–9]. Thus, the expression level of inactivating cholinesterase differs substantially between both systems. Neuronal acetylcholine is rapidly hydrolyzed allowing signalling on the millisecond time scale, whereas non-neuronal acetylcholine acts in an auto- and paracrine way for longer time periods.

Importantly, one has to consider that any condition which modifies either the spontaneous hydrolysis of acetylcholine or the activity of the specific acetylcholinesterase (E.C. 3.1.1.7) or the unspecific butyrylcholinesterase (EC 3.1.1.8) will cause substantial side effects on the auto-paracrine action of non-neuronal acetylcholine. It is known that spontaneous hydrolysis of acetylcholine is pH-dependent, thus leaving acetylcholine conserved at acidic pH below 6.0. Moreover, both esterases are acid-labile enzymes showing a pH-optimum at pH 7.6 for the specific esterase. The unspecific butyrylcholinesterase is somewhat less pH-dependent and operates within a range of 6–8 (see documentation Sigma Aldrich; [10]). The present paper describes consequences of a reduced extracellular pH with respect to non-neuronal acetylcholine.

2. Methods

2.1. Hydrolysis of acetylcholine by acetylcholinesterase and butyrylcholinesterase at pH 4.3 and 7.0

Buffer media with pH 4.3 and 7.0 were prepared by use of monobasic sodium phosphate (0.4 M) or a combination of 0.2 M monobasic (39 ml) and 0.2 M dibasic (61 ml) sodium phosphate. Acetylcholine was added to obtain final concentrations of 5 or 25 mM or a lower concentration of 2 μM. In addition, 1.5 or 10 units of acetyl- or butyrylcholinesterase were added to 1 ml aliquots of the buffer media. Thereafter, these samples were stored at 36 °C and 10 μl aliquots were removed at the start and

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end of the incubation period. Acetylcholine was measured by HPLC combined with enzyme reactors as described elsewhere [2].

2.2. Non-invasive assay of non-neuronal acetylcholine from the human skin

This pilot investigation was performed with three volunteers. A gauze bandage (5 cm wide and 100 cm long) was soaked in 25 ml water and placed for 20 min on the forearm; these experiments had been approved by the ethical review board. The gauze bandage was then removed and placed in a 25 ml syringe to collect the fluid remaining within the gauze bandage after being in contact with the human skin. The fluid was filtered and an aliquot of 20 μl was injected into the HPLC-system (70 mM phosphate buffer with 0.3 mM EDTA; pH 8.5 adjuste). A cationic exchange high-pressure liquid chromatography (HPLC) combined with bio-reactor and electrochemical detection was used [2].

3. Results

3.1. pH-dependency of esterase activity

Fig. 1 demonstrates that at a pH of 4.3 even a concentration of 5 mM acetylcholine was not at all hydrolyzed by 10 U of the specific acetylcholinesterase after 60 min. One unit of esterase will hydrolyze 1 μmol acetylcholine in 1 min at a pH of 8.0 [11]. Thus, in the present experiments with 1 ml of a 5 mM acetylcholine solution (i.e. containing 5 μmol acetylcholine) 10 U esterase should inactivate all acetylcholine within 30 s at a physiological pH of 7.4, which represents more or less the optimum pH for this enzyme (7.60; see Reference 11). When the experiments were repeated with the same concentration of acetylcholine or with a considerably lower acetylcholine concentration (2 μM) about 15% acetylcholine were hydrolyzed within 60 min (not shown).

The butyrylcholinesterase is somewhat less pH-dependent, with about 20–30% of acetylcholine being hydrolyzed to choline within 60 min at a concentration of 5 mM (see Fig. 1) or 2 μM acetylcholine (not shown). The high enzymatic capacity of both enzymes is demonstrated in Fig. 2, showing the results when a pH of 7.0 was tested; 25 μmol acetylcholine was completely inactivated by 1.5 U of either enzyme. This extreme difference to the situation at a pH of 4.3 gives some impression about physiological or pathophysiological mechanisms which have to be considered when the pH value falls below 7.

3.2. Non-invasive assay for non-neuronal acetylcholine from the human skin

After 20 min exposure to the skin about 2–4 ml water was recovered. Upon injection of an aliquot of the collected fluid into the HPLC, we found a peak with a retention time corresponding to the acetylcholine standard (Fig. 3). The same probe was spiked with a small quantity of exogenous acetylcholine and the corresponding single peak increased (Fig. 3) thus confirming the presence of acetylcholine in the sample. This was further demonstrated by injecting the same sample into the HPLC system and using an acetylcholinesterase-packed analytical column. Under this condition the respective acetylcholine peak completely disappeared, which can be regarded as proof of the existence of acetylcholine. Fig. 3 shows the results obtained from a female and male volunteer.

4. Discussion

The cellular effect of neuronally released acetylcholine is limited within millisecond based on the extreme high turnover-rate of the hydrolyzing enzyme acetylcholinesterase (5000 molecules of acetylcholine/enzyme/s) and the high expression of the enzyme close to neurons and

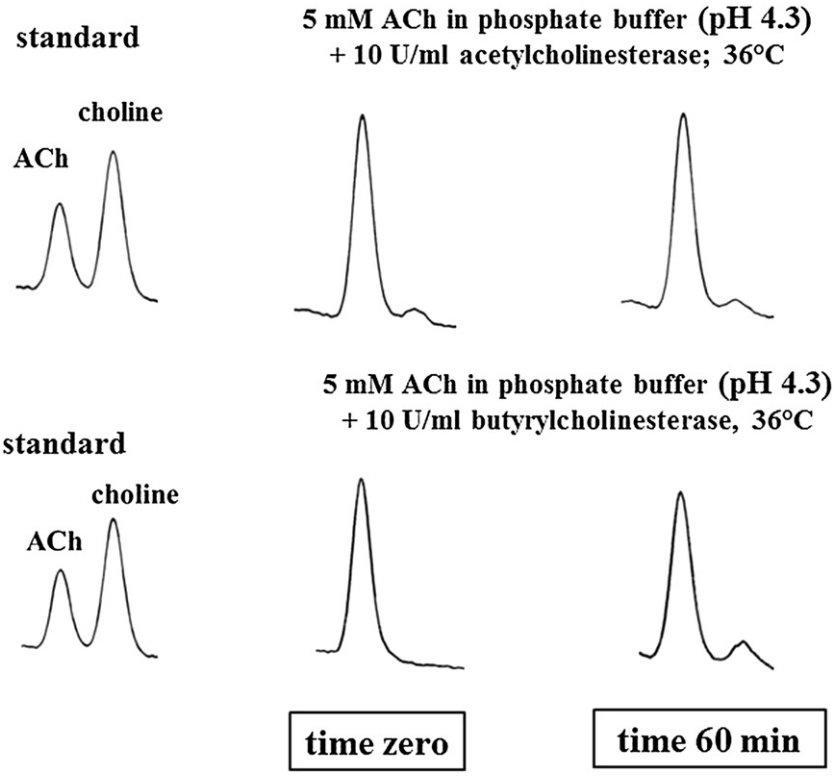

Fig. 1. Low esterase activity at a pH of 4.3. On the left hand side the chromatograms of 1 pmol/20 μl of acetylcholine (ACh) and choline are shown. A phosphate buffer with a pH of 4.3 was used to prepare a 5 mM acetylcholine solution (1 ml) containing additionally 10 U of either acetylcholinesterase or butyryl-cholinesterase. Aliquots of 10 μl were removed at the start and end of a 60 min incubation period at 36 °C for measurement of acetylcholine and choline. A new stock solution of acetylcholine was used when the activity of butyrylcholinesterase was tested.