A NEUROTRANSMITTER'S (ACETYL CHOLINE) IN-VITRO OXIDATION AND ITS KINETIC STUDY BY USING CHLORAMINE-T(AN INFECTICIDAL AGENT) AS AN OXIDANT

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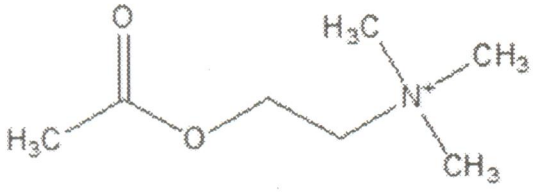
ABSTRACT

Acetylcholine and its analogs slow down the heart rate by producing the hyperpolarization in the pacemaker cells. Major effect of acetylcholine on heart function is to regulate its function by decreasing the heart rate. The enzyme acetylcholinesterase diminishes its effect and breaks down acetylcholine into the inactive metabolites choline and acetate. Present project deals with the kinetic and mechanistic study of breakdown (oxidation) of acetylcholine chloride by chloramine-T in $HClO_4$ medium, which is spectrophotometrically monitored at λ_{max} of 256 nm. The dependencies of the reaction rate on acetylcholine chloride and the reduction product of CAT, *p*-toluenesulfonamide, were studied. A mechanism consistent with the observed kinetic data has been proposed and discussed.

INTRODUCTION

The compound acetylcholine, often abbreviated as AC, was the first neurotransmitter to be identified. It is a chemical transmitter in both the peripheral nervous system (PNS) and central nervous system (CNS) in many organisms including humans.

Acetylcholine is a methyl ester of choline with the following chemical formula $CH_3COOCH_2CH_2N+(CH_3)_3$ and structure:



This structure is reflected in the systematic name, *2-(acetyloxy)-N,N,N-trimethylethan* aminium.

Acetylcholine (AC) was first identified in 1914 by Henry Hallett Dale for its actions on heart tissue. It was confirmed as a neurotransmitter by Otto Loewi[1] who initially gave it the name vagusstoff because it was released from the vagus nerve. Both received the 1936 Nobel Prize in Physiology and Medicine for their work. Later work showed that acetylcholine binding to acetylcholine receptors on striated muscle fibers, opened channels in the membrane. Sodium ions then enter the muscle cell, stimulating muscle contraction. It is an essential nutrient to improve the physical performance. Acetylcholine (AC) on enzymatic hydrolysis forms acetic acid and choline[2], which plays a significant role in the functioning of important organs, such as the heart, brain, reproductory system, and liver.[3]

"Acetylcholine is released at the vagal endings by the stimulation of the parasympathetic nerves to the heart. It has two major effects on the heart. First it decreases the rhythms of the sinus node, and second, it decreases excitability of the A-V junctional fibers between atrial musculature and A-V node, thereby slowing the transmission of the cardiac impulse into the ventricles. Moreover it greatly increases permeability of the fiber membranes to potassium ions, which allows rapid leak of potassium ions out of the conductive fiber and causes hyperpolarization. This decreases the resting membrane potential of the sinus node fibers to -65 to -75 millivolts rather than -55 to -60 millivolts[15]".

Although acetylcholine induces contraction of skeletal muscle, it acts via a different type of receptor (muscarinic) to *inhibit* contraction of cardiac muscle fibers. M2 receptors are located mainly in the supraventricular parts of the heart. M2 muscarinic receptors, in contrast to M1 and M3 receptors, tend to mediate inhibition of cellular activity. They do so

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through G proteins that inhibit adenylyl cyclase (opposite of the activation of adenylyl by beta adrenergic receptors) and by activation of K channels in the plasma membrane. Clinically important examples of K channel activation by ACh are especially prominent in the supraventricular parts of the heart. Thus, it is important to be familiar with the effects of ACh on the cellular electrophysiology of the heart[8].

Normally, the enzyme acetylcholinesterase converts acetylcholine into the inactive metabolites, choline and acetate. The devastating effects of nerve agents are due to their inhibition of this enzyme, resulting in continuous stimulation of the muscles, glands and central nervous system[46].

Certain insecticides are effective because they inhibit this enzyme in insects. On the other hand, since a shortage acetylcholine in the brain has been associated with Alzheimer's disease, some drugs that inhibit acetylcholinesterase are used in the treatment of that disease[5] can diffuse into the eye. It is sold by the trade name Miochol-E (CIBA Vision). Similar drugs are used to induce mydriasis (dilation of the pupil) in cardiopulmonary resuscitation and many other situations[6].

The disease myasthenia gravis, characterized by muscle weakness and fatigue, occurs when the body inappropriately produces antibodies against acetylcholine receptors, and thus inhibits proper acetylcholine signal transmission. Drugs that competitively inhibit acetylcholinesterase (e.g., neostigmine or physostigmine) are effective in treating this disorder.

Blocking, hindering or mimicking the action of acetylcholine has many uses in medicine. Cholinesterase inhibitors increase the action of acetylcholine by delaying its degradation; some have been used as nerve agents or pesticides. Clinically they are used to reverse the action of muscle relaxants, to treat myasthenia gravis and in Alzheimer's disease[7].

Acetylcholine Chloride

Acetylcholine chloride molecular formula $CH_3CO_2CH_2CH_2N(CH_3)_3Cl$ M.W.(181.66), a chloride salt of acetylcholine is a synthetic version of the central and peripheral neurotransmitter. But it is also used as a vasodialtor and a cardiovascular agent. Available via prescription, actylcholine chloride is used to keep eye pupils dilated during eye surgery and as a bathing solution for the eyes[8].

Acetylcholine chloride solution is utilized in cataract surgery and penetrating keratoplasty. Administrated by interocular irrigation, acetylcholine chloride promotes rapid and complete miosis, or constriction of the pupil of the eye. It is also employed as a pharmaceutical raw material for creating other prescription drugs[9].

S. Zhang et al[10]. while studying the affect of organphosphorous pesticides on living organisms used the enzyme choline oxidase along with acetylcholineestrase for the oxidation of the choline.

It is obvious from the its uses and its presence *in vivo* and *in vitro* that more knowledge and understanding about the oxidative behavior of acetylcholine choride is useful as it can further be used for the benefit of humankind.

Chloramine-T

Chloramines can be used as bleach, disinfectants and oxidants. Organic disinfectants slowly gives off chlorine, causing a slower and less aggressive disinfection than with hypochlorite (OCl^-). Chloramines can be used to improve odor and flavor of the water when chlorine is used as a disinfectant[11].

They have diverse properties and behave both as oxidizing and halogenating agents. They are also used as analytical reagents in determining a variety of reductants in solutions[12].

The most important members of this class of compounds are chloramine-T (CAT). The overall reaction for monochloramines involving a two-electron change can be written as,

$$ArNC1Na + 2I^- + 2H^+ \longrightarrow ArNH_2 + I_2 + Na^+ + Cl^-$$

Due to the diversity of applications of chloramines-T for multiple purposes and its ability as a versatile oxidizing agent, we have tried to study the kinetics of the oxidation of other one biologically important compounds namely acetylcholinechloride.

It is obvious from the their uses and their presence in vivo and in vitro that more knowledge and understanding about acetylcholine choride is useful as it can further be used for the benefit of humankind.

MATERIAL AND METHOD

Setting:

This research was performed at Western Illinois University U.S.A. in 2003 by the author.

(Shimadzu UV-1601) UV/Visible high performance spectrophotometer features include photometric, kinetic, spectrum scanning, multiwavelength, and quantization capabilities.

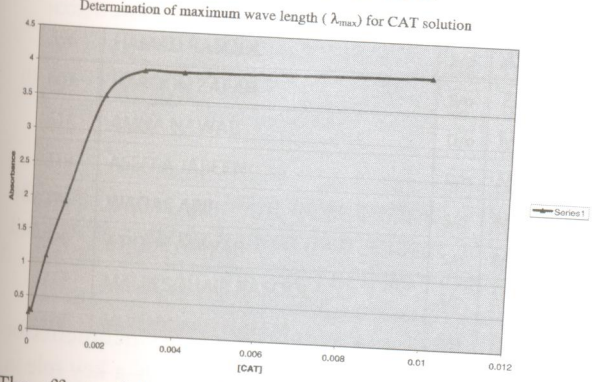
In the spectrum mode, plots of absorbance *vs* wavelength are taken to determine the λ_{max} value. 11.7M $HClO_4$ (fisher Chemical Co), 0.1 M $HClO_4$, 0.1 N NaOH, 0.05 N KIO_3 solution, 0.05 N $Na_2S_2O_3$, 0.05 M CAT, Acetylcholine chloride 0.1 M.

As it is shown in fig .(1) 256 nm was selected as λ_{max} for monitoring CAT in the reaction mixture.

RESULTS

Determination of effect of acid

Fig-1 : A plot of absorbance vs. [CAT] shows that the Beer's law is obeyed in the range, 0-2.00 x 10^{-3} M CAT. Therefore the concentration of CAT in the kinetic run was kept below 2.00 x 10^{-3} M



The effect of the acid was observed in oxidation of AC by CAT by varying the acid concentration of the reaction mixture and the change in absorbance vs time elapsed had been monitored for each run ln Absorbance vs time was ploted and the rate constant k was determined by calculating the slope of the plot.

The order of reaction was obtained by plotting A Plot of lnk vs ln $[HClO_4]$ and order reaction was found to be 0.5.

A Plot of lnk vs ln $[HClO_4]$ gave a straight line (Fig.2) with slope of 0.5 which gives the order of reaction at the various $[HClO_4]$.

Dependence of the rate on [CAT]

$HClO_4$ 0.500 M, 4.00 x 10^{-2} M AC, λ_{max} 256 nm, Temperature = 50°C

Table-1 : Kinetic data for the effect of HClO4 1.588 10^{-3} M CAT, 4 x 10^{-2} M AC, Temperature 50°C

[HClO4]	Ln [HClO4]	k s-1	Ln k
0.07	-2.659	8.034× 10 –6	-11.73
0.1	-2.302	1.084× 10 –5	-11.43
0.3	-1.204	2.107× 10 -5	-10.77
0.5	-0.693	2.461× 10 –5	-10.61
0.7	-0.357	2.508× 10 –5	-10.59
1	0.0000	2.921× 10 –5	-10.44

Fig-2 : A Plot of lnk vs ln $[HClO_4]$ gave a straight line with slope of 0.5,which is the order on $[HClO_4]$

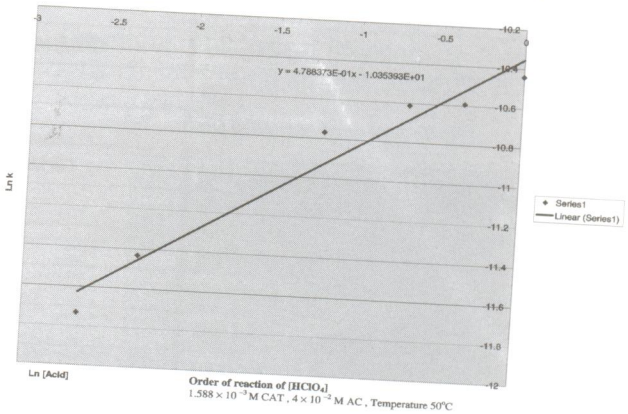
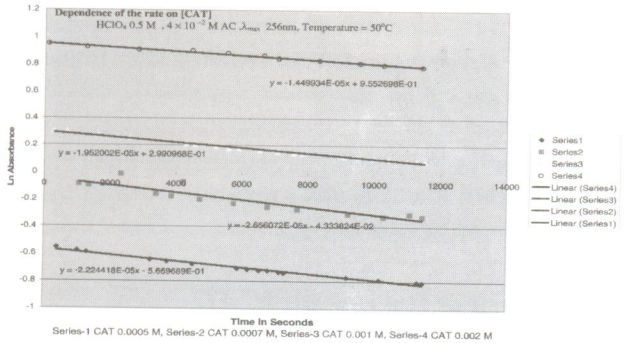


Table-2 : Data for [CAT] effect

[CAT] M	k (s^{-1})
1.000 × 10^{-3}	1.952× 10^{-5}
1.558 × 10^{-3}	2.461× 10^{-5}
2.000 × 10^{-3}	1.449× 10^{-5}
5.000 × 10^{-4}	2.224× 10^{-5}
7.000 × 10^{-4}	2.656× 10^{-5}

Fig-3 : A plot of varying [CAT]



Series-1 CAT 0.0005 M, Series-2 CAT 0.0007 M, Series-3 CAT 0.001 M, Series-4 CAT 0.002 M

The near constancy of k values at different [CAT] in Table 35 shows that the reaction follows a first-order dependence on [CAT].

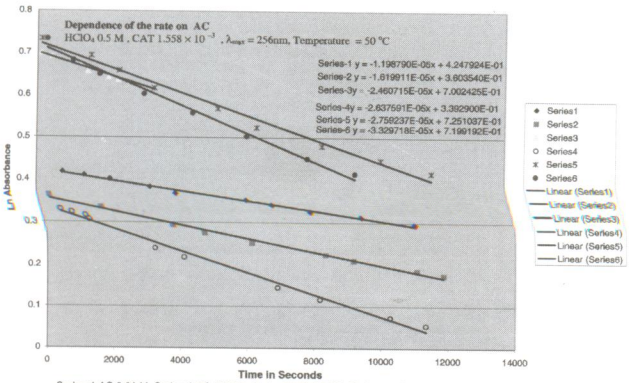
Determination of the effect of [Acetylcholine chloride]

The effect of different AC concentrate ions on the rate constant k had been determined by varying the

Table-3 : Temperature = 50°C

[AC]	Ln [AC]	K(s^{-1})	ln k
1.00×10^{-2}	-4.605	1.199×10^{-5}	-11.33
2.00×10^{-2}	-3.912	1.620×10^{-5}	-11.03
4.00×10^{-2}	-3.218	2.461×10^{-5}	-10.61
6.00×10^{-2}	-2.813	2.637×10^{-5}	-10.54
8.00×10^{-2}	-2.526	2.759×10^{-5}	-10.49
1.20×10^{-1}	-2.120	3.329×10^{-5}	-10.31

Fig-4 : A plot of varying [AC]



Series-1 AC 0.01 M, Series-2 AC 0.02 M, Series-3 AC 0.04 M, Series-4 AC 0.06 M, Series-5 AC 0.08 M, Series-6 AC 0.12 M.

concentration of acetylcholine chloride while keeping the others constant i.e $HClO_4$ 0.5 M, CAT 1.558×10^{-3}, λ_{max} = 256nm.

The order of reaction on [AC] was found by plotting ln k' *vs* ln [AC] where slope gave the order. The order of reaction found to be the 0.5

DISCUSSION

With the substrate in excess, at constant [$HClO_4$], [AC]^o and temperature, the [CAT]^o was varied. Plots of ln [CAT] *vs*. time were linear indicating a first-order dependence of the rate on [CAT]^o. The pseudo first-order rate constants (k') calculated from the slopes are given in Table 2. The values of *k* are unaltered with variation of [CAT]^o confirming the first-order dependence of the rate on [oxidant]^o.

When [AC]^o was varied, keeping all other conditions the same, the rate increased with increase in [AC]$_o$ (Table 3) and a plot of ln *k vs*. ln [AC]^o was linear (r = 0.9999) with a slope of 0.5 indicating a fractional-order dependence on [AC]^o. Furthermore, a plot of ln k *vs*. ln [AC]^o was linear (r = 0.9880) with a y-intercept, confirming the fractional-order dependence on [AC]^o. Similarly, the rate of reaction increased with increasing [$HClO_4$] (Table 1) and a plot of log *k vs*. log [$HClO_4$] was linear (r = 0.9995) with a slope of 0.5 indicating a fractional-order dependence of the rate on [H^+].

Investigations under the conditions [CAT]$_o$ >> [AC]$_o$ revealed the following stoichiometric reaction:
where Ar = *p*-Me-C_6H_6-SO_2

Rate = $\{K_1K_2k_3\,[CAT]_t\,[S]\,[H^+]\} / \{1 + K_1[H^+] + K_2[S]\}$

$$\underset{\text{(AC)}}{CH_3CO_2CH_2CH_2\overset{+}{N}(CH_3)_3} + \underset{\text{(CAT)}}{2ArNClNa} + 2H_2O \longrightarrow \underset{\text{(protonated Betaine)}}{(CH_3)_3\overset{+}{N}CH_2COOH} + CH_3COOH + \underset{\text{(PTS)}}{2ArNH_2} + 2NaCl \qquad (1)$$

The rate law derived is in good agreement with the experimental results such as first order in [CAT] and fractional order each in [AC] and [H^+].

"Acetylcholine (AC) kinetics in neuroeffector junctions (NEJ) of the sinus node plays a key role in vagal control of heart rate. Prior studies have shown that the concentration of AC in NEJ appears to follow first-order linear kinetics. Earlier studies showed that [AC] follows first-order linear kinetics, because they predicted [AC] only at pacemaker cells. AC kinetics at other sites in the NEJ, such as at nerve endings, is

different"[14].

Our results match with the study done in vivo i.e, rate order is first order with respect to [CAT] but it is linear and fractional with respect to [H^+]. We assume that depending upon the physiological pH of the site its kinetics may be different than at neuroeffector junctions (NEJ). This is an important finding because by assuming that acetylcholine dissociation at lower physiological pH is lesser, we can predict that at sinus node it can prolong the hyperploarization with more negative value i.e., -65millivolts to -75 millivolts. Which can further prolong the effect of stimulation of vagal nerve to the heart. So a moderate decrease in pH at heart surroundings may simply extra increase the delay of conduction impulses. So we can relate the physiological pH of the heart with the bradycardia in terms of breakdown of acetylcholine. A further study in detail might be helpful in understanding more about bradycardia, hypotension, hypersecretion, brochoconstriction, GI tract hypermotility, and decrease intraocular pressure because of acetylcholine important role in all these places.

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