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Research Article

EFFECT OF VERY HIGH DILUTION OF ACETYLCHOLINE ON ISOLATED FROG'S HEART

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ABSTRACT

In the Homoeopathic system of medicine, drugs are used both in low as well as very high dilutions. The very high dilution does not contain any active molecule of the dissolved substance, when calculated according to Avogadro's hypothesis (non molecular high dilutions). As such dilutions without any molecule of the dissolved substance should not induce any action. The present study was conducted to assess the efficacy of very high dilutions of acetylcholine (Ach) on cardiac tissue of *Rana Tigrina*. Ach is a ubiquitous neurotransmitter; exert an inhibitory effect on cardiac muscle through M_2 receptor. The Ach dilutions (1C to 200C) were prepared in three ways: by simple mixing without giving succusion (N-dilutions), by "dynamisation" method with manual succusion (D-dilutions) and dynamised water without adding Ach (D-water). Fourteen dilutions of ach (N-dilutions, D-dilutions and D-water) were randomly selected and tested on each isolated preparation of frog's heart. The action of each Ach dilution on the normal rhythm of frog's heart was recorded. The N-dilutions of Ach induced an inhibitory effect on the normal rhythm of heart, only up to the scale of 10C. The D-dilutions of Ach induced effect up to the scale of 200C. The D-dilutions of Ach exerted a significant inhibitory effect at 2C (p<0.001), 5C (p<0.01), 10C (p<0.001), 15C (p<0.001), 50C (p<0.001) and 200C (p<0.001). We conclude that very high dilutions of Ach were effective in inducing dynamic effect on normal rhythm of Frog's heart. Further studies are directed towards finding out the sub molecular structure in the solvent.

Keywords: Acetylcholine, Dynamisation, High dilution

INTRODUCTION

In the Homoeopathic system of medicine, drugs are used both in low as well as very high dilutions. The very high dilution does not contain any active molecule of the dissolved substance, when calculated according to Avogadro's hypothesis (non molecular high dilutions). As such dilutions without any molecule of the dissolved substance should not induce any action. But studies have shown that high dilutions are more potent in action than low dilutions[1-3]. Moreover, researchers believe that during preparation of high dilutions, certain form of energy is transferred from the drug to solvent[4]. To assess the efficacy of non-molecular high dilutions, we endeavored to study an effect of acetylcholine (Ach) in various high dilutions on cardiac tissue of *Rana Tigrina*. Ach exert an inhibitory effect on cardiac muscle through M_2 receptor[5].

Ach is a ubiquitous neurotransmitter. It is the neurotransmitter of parasympathetic nervous system, preganglionic fibers of sympathetic nervous system, neuromuscular junction and some neurons in the central nervous system. Ach plays numerous roles. In the brain, Ach is involved in excitability, arousal, reward systems, learning and memory. Ach also regulates and activates muscle movement and sweat gland function[6].

MATERIALS AND METHOD

Drugs and dilutions

Acetylcholine chloride (S.D.Fine chemicals Ltd, Boisar, India) was progressively diluted with water at a geometrical ratio of 1:100 (centesimal dilutions). 0.1 mg of Ach was mixed with 9.9 ml of water to prepare 10ml of first Ach dilution at 10^{-2} dilution factor expressed as 1C. The second dilution 2C at 10^{-4} dilution factor was prepared by taking 0.1ml of 1C Ach and 9.9 ml of water. Thus, a scale of centesimal dilutions between the first dilution 1C (10^{-2}) and the 200th dilution 200C (10^{-400}), with a content of 6.023×10^{18} and 6.023×10^{-380} molecules per 100ml respectively was obtained, from which fourteen dilutions were selected randomly. The number of Ach molecules/100ml of dilution was calculated according to the Avogadro's number (6.023×10^{23} molecules/mol), considering molecular weight of acetylcholine chloride is 181.5g-200g (Table-1)[7].

Table 1: Content of fourteen centesimal dilutions calculated in moles and molecules of acetylcholine /100ml, according to molecular weight and Avogadro's number.

Dilutions of acetylcholine		Mol %	Molecules/100ml
Centesimal Scale	**Dilution factor**		
2C	10^{-4}	5×10^{-7}M	6.023×10^{16}
5C	10^{-10}	5×10^{-13}M	6.023×10^{10}
10C	10^{-20}	5×10^{-23}M	6.023×1
12C	10^{-24}	5×10^{-27}M	6.023×10^{-4}
15C	10^{-30}	5×10^{-33}M	6.023×10^{-10}
20C	10^{-40}	5×10^{-43}M	6.023×10^{-20}
25C	10^{-50}	5×10^{-53}M	6.023×10^{-30}
50C	10^{-100}	5×10^{-103}M	6.023×10^{-80}
75C	10^{-150}	5×10^{-153}M	6.023×10^{-130}
100C	10^{-200}	5×10^{-203}M	6.023×10^{-180}
125C	10^{-250}	5×10^{-253}M	6.023×10^{-230}
150C	10^{-300}	5×10^{-303}M	6.023×10^{-280}
175C	10^{-350}	5×10^{-353}M	6.023×10^{-320}
200C	10^{-400}	5×10^{-403}M	6.023×10^{-380}

Note: Centesimal dilutions from 2C to 10C are molecular dilutions where as 11C to 200C are non molecular dilutions as per Avogadro's hypothesis.

The dilutions were prepared in three ways

(a) By simple mixing, without giving succusion (N-dilutions) ,

(b) By "dynamisation" method with manual succusion (knocking slightly 10 times the vial against a firm substrate, rhythmically at a frequency of one succussion per second for each dilution) according to Hahnemann's Homoeopathic technique (D-dilutions)[8,9]

(c) Dynamised water (D-water) was prepared by giving manual succusion without adding Ach in it.

Animals and Technique

Six laboratory bred adult male *Rana tigrina* frogs weighing 200 ± 5gm were selected. They were sacrificed by using pithing needle and puncturing spinal cord through it. The isolated frog's heart was mounted on Symes canula, which was connected to a jar containing fresh Ringer's solution. The head flow of Ringer's solution was maintained constant throughout the experiment. The motor activity (motility) of the isolated organ was recorded by using a Kymograph[10]. All the randomly selected fourteen dilutions of Ach (N-dilutions, D-dilutions and D-water) were tested on each isolated preparation of frog's heart. The action of Ach dilution at various doses on the normal rhythm of frog's heart was recorded. 100µl of different dilutions of Ach was dropped with a pipette in to the canula for the purpose. New pipette tips were used for each dilution. The amplitude of contraction before and after test dose was expressed in centimeters. An interval of time between two tests was given to regain normal rhythmicity of heart.

The entire experimental protocol was approved by institutional ethical committee. Mean ± SD values were calculated for each dilution on 6 preparations. The Student 't' test was applied to determine the statistical significance. Statistical significance was established at $p < 0.05$[11].

RESULTS

Table-2 shows that the N-dilutions of Ach have induced an inhibitory effect on the normal rhythm of heart, only up to the scale of 10C.

The D-dilutions of Ach exerted variable dynamic effect up to the scale of 200C with a content of 6.023×10^{-380} molecules/100ml (Table-3). The D-dilutions of Ach exerted a significant inhibitory effect at 2C ($p<0.001$), 5C ($p<0.01$), 10C ($p<0.05$), 15C ($p<0.001$), 50C ($p<0.001$) and 200C ($p<0.001$).

D-water exerted no effect on cardiac contractility.

Table 2: Effect of N-Dilutions of acetylcholine at various doses on isolated Frog's heart.

N-Dilutions of Ach	Amplitude of contraction	
	Before adding Ach (cm)	After adding Ach (cm)
2C	3.58 ± 0.36	0.34 ± 0.18***
5C	3.64 ± 0.31	2.48 ± 0.14*
10C	3.62 ± 0.30	2.88 ± 0.41*
12C	3.54 ± 0.33	3.54 ± 0.31^{NS}
15C	3.50 ± 0.31	3.50 ± 0.31^{NS}
20C	3.55 ± 0.34	3.55 ± 0.34^{NS}
25C	3.53 ± 0.32	3.53 ± 0.32^{NS}
50C	3.61 ± 0.30	3.61 ± 0.30^{NS}
75C	3.56 ± 0.33	3.56 ± 0.33^{NS}
100C	3.58 ± 0.34	3.58 ± 0.34^{NS}
125C	3.54 ± 0.33	3.54 ± 0.33^{NS}
150C	3.62 ± 0.35	3.62 ± 0.35^{NS}
175C	3.57 ± 0.37	3.57 ± 0.37^{NS}
200C	3.58 ± 0.36	3.58 ± 0.36^{NS}

Values are given in Mean ± SD; Ach-acetylcholine, cm-centimeter; *$p<0.05$, **$p<0.01$, ***$p<0.001$, ^{NS} Not significant.

Table 3: Effect of D-dilutions of acetylcholine on isolated Frog's Heart.

D-Dilutions of Ach	Amplitude of contraction	
	Before adding Ach (cm)	After adding Ach (cm)
2C	3.50 ± 0.38	0.27 ± 0.19***
5C	3.64 ± 0.31	3.11 ± 0.14**
10C	3.62 ± 0.30	3.62 ± 0.41^{NS}
12C	3.51 ± 0.39	3.51 ± 0.40^{NS}
15C	3.61 ± 0.31	0.20 ± 0.11***
20C	3.55 ± 0.34	3.52 ± 0.32^{NS}
25C	3.53 ± 0.32	3.53 ± 0.32^{NS}
50C	3.55 ± 0.32	0.14 ± 0.08***
75C	3.58 ± 0.32	3.57 ± 0.31^{NS}
100C	3.55 ± 0.32	3.55 ± 0.32^{NS}
125C	3.55 ± 0.33	3.52 ± 0.33^{NS}
150C	3.62 ± 0.35	3.61 ± 0.35^{NS}
175C	3.56 ± 0.37	3.55 ± 0.37^{NS}
200C	3.51 ± 0.32	0.14 ± 0.10***

Values are given in Mean ± SD; Ach-Acetylcholine, cm-centimeter; *$p<0.05$, **$p<0.01$, ***$p<0.001$, ^{NS} Not significant

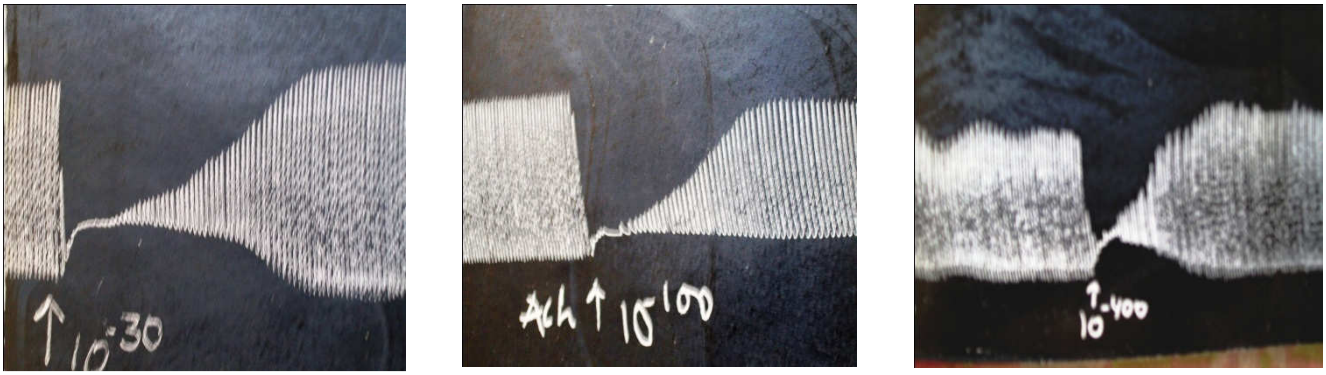
DISCUSSION

The results of the present study represents dynamised dilutions (D) of Ach prepared by Hahnemann's technique and non-dynamised dilutions (N) of Ach had exerted both quantitative and qualitative differences in their action on isolated frog's heart.

Qualitative difference: At the same degree of dilution, the D-dilutions had a more intense biological effect than the N-dilutions (wide Table 2 &3).

Quantitative difference: The N-dilutions exerted effect only up to the limit of the molecular specifity, calculated on the basis of Avogadro's number at 10C dilution, with a content of 6.023×10^{1} molecules of

Ach/100ml. In contrast, the D-dilutions exerted a statistically significant biological effect at high dilutions, over 11C, which mathematically and according to Avogadro's number, do not contain any molecule of the dissolved substance (Fig 1).



(a) **(b)** **(c)**

Fig. 1: Shows the inhibitory action of D-dilutions at 15C (a), 50C (b) and 200C (c) on normal rhythm of frog's heart.

The efficacy of N-dilutions decreased where as D-dilutions increased with an increase in dilution. This indicates that higher dilutions prepared by dynamisation were more dynamic when compared with dilutions prepared by simply mixing. But it is difficult to explain the action of non-molecular high dilution beyond 11C scale at the receptor level due to the absence of ligand or informative molecule. A majority of informative molecules or ligand interact with their receptors according to the action of mass law[12]. N-dilutions exerted effect only up to the presence of ligand in it, where as D-dilutions exerted effect, even after the absence of ligand. This represents that D-dilutions contain some other form of information which is absent in the N-dilutions. The D-dilutions might have obtained this new form of information during the process of dynamisation, by collision of molecules of dissolved substance and solvent. So, the information in high D-dilutions should be looked for another informational substrate may be a sub molecular structure, a certain form of energy or merely a specific molecular organization of the solvent[4].

A study by NMR spectroscopy demonstrated chemicophysical changes in high diluted aqueous solutions induced through succusion[13,14]. Recently, a study conducted by Chikramane et al. by Transmission Electron Microscopy (TEM), electron diffraction and chemical analysis by inductively coupled Plasma-Atomic Emission Spectroscopy demonstrated that the dissolved substance (starting substance) retains in high dilutions in the form of nanoparticles[15].

The duration of action has not been noted but as per our observation, the D-dilutions have acted for longer duration than N-dilutions. The cardiac tissue has shown a selective response to Ach at particular concentrations only. This may be due to presence of specific receptors for Ach at different concentrations. The reason for this has to be ruled out in the further studies.

We conclude from the present study that very high dilutions of Ach are effective in inducing dynamic effect on normal rhythm of frog's heart. A selective response from cardiac tissue to Ach at particular concentrations was observed. Further studies are directed towards finding out the sub molecular structure in the very high dilution.

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