MECHANISM OF VARIATION IN THE ACTION OF ACETYLCHOLINE IN ISOLATED FROG HEARTS

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Summary: Actions of increasing concentrations of acetylchlione (Ach) on the electrical (ECG) and mechanical (cardiac output) activity of isolated frog hearts were investigated. Ach acted at three different sites: (i) Pacemaker (ii) conduction of impulse (iii) cardiac musculature. The changes in electrical parameters of ECG had no specific relationship with change in mechanical response. In 21 (60%) hearts typical dose dependent inhibitory action was observed while in 14 (40%) hearts biphasic action or its variants like stabilization or reduction or absense of inhibitory action or clear excitatory action was observed at intermediate concentrations between the minimum effective (10^{-9} *g/ml*) and asystolic (10^{-7} to 10^{-5} *g/ml*) strengths. Biphasic actions were common in summer. It is suggested that this variability of action of Ach may be due to the presence of separate excitation and inhibition coupled cholinergic receptors or processes and their interaction.

Key words: sites of Ach action biphasic Ach action excitation-inhibition coupled receptors cholinergic receptors ECG and mechanical activity

Work on the actions of increasing concentrations of acetylcholine (Ach) on different parameters of cardiac activity (i.e. cardiac output, arterial pressures and heart rate) under constant perfusion pressure and fluid inflow, has already been published (2,3). The concentration-response curves recorded in that work suggested a decrease in action of Ach in some hearts at intermediate concentrations between the minimum effective (threshold) and stopping (asystolic) concentrations.

It was considered essential to extend this work by recording mechanical and electrical activity simultaneously in order to determine the sites of action and mechanism of variation in the action of Ach at different concentrations.

MATERIALS AND METHODS

The frog heart was perfused through a cannula introduced in the posterior vena cava. Ringer's solution from a main perfusion reservoir continuously flowed into the heart at a constant pressure of 22 mm of water and at a constant flow rate (both regulated by an overflow device) so as to avoid cardiodynamic changes due to change in pressure (10,11). Test solutions of Ach were prepared in the same sample of Ringer's solution which was used for the perfusion of the heart. The concentration of 10^{-3} *g/ml* was prepared by dissolving 10 *mg* of solid Ach chloride in 10 *ml*

of Ringer's solution. Due precautions were observed in the weighing of solid Ach chloride to minimise contact with moisture. The other strengths ranging from 10^{-4} to 10^{-9} *g/ml* (or still lower concentrations when necessary) were prepared by a standard dilution procedure in clean sterile 100 *ml* volumetric flasks. Test solutions were administered by perfusion from a different reservoir, but through the over-flow to device to eliminate changes in perfusion pressure and flow rate while changing the perfusion from one source to the other.

The mechanical and electrical activity was recorded electronically using a two channel ink writing recorder. The electrocardiogram was recorded on one channel while the other channel recorded the cardiac output in drops. The output from the aortae entered a glass chamber from which the fluid flowed out in drops of uniform size. Each drop was converted into an electrical signal with the help of a modified photo-transister drop recording assembly (14). The output from the second channel was also passed through an audioamplifier and loud speaker to provide an audible indication of drops. A trigger valve on the drop-recording assembly also provided visual indication of drop-impulses. The two channels were simultaneously displayed on a double beam oscilloscope for detailed analysis. The cardiac output in ml/minute was calibrated by measuring the volume of drops collected over one minute in a 10 *ml* graduated cylinder.

The pH of Ringer's solution and one of the concentrated solutions of Ach was recorded with the help of Photovolt pH meter. All test and control solutions were allowed to stand at a common place near the heart so as to permit equalization of temperature. The experiments were conducted in the months of May and June at room temperature.

RESULTS

The results of experiments on 35 hearts are considered here. Increasing concentrations of Ach were tested in each heart and a continuous record was obtained starting from the minimum effective concentration and working up to the asystolic concentration. The action of each concentration was bracketed between normal records on either side as shown in the sample records of two hearts in Fig. 1A, one showing the action of Ach 10^{-8} *g/ml* (tracing A), and the other showing the action of asystolic concentrations (tracing B). The actions of Ach on mechanical and electrical activity are considered below:—

Mechanical Activity: In 21 (60%) hearts increasing inhibitory effects were observed on the mechanical activity as the concentration of Ach was increased. Both the cardiac output and heart rate were inhibited. Where the heart rate was unaltered the change in the cardiac out put indicated change in the inotropic response due to the action of Ach on ventricular muscle. In the remaining 14(40%) hearts various unusual concentration-response patterns (Fig 2) having the following common features, were observed.

(1) Clear biphasic effects i.e. stimulation at some concentration and inhibition at the other concentration. The minimum effective concentration always produced inhibitory action, suggesting that the inhibition-coupled receptors were more sensitive

to the action of Ach. The excitatory action was prominent on the chronotropic response (pacemaker) as well as on ventricular musculature.

(2) Stabilization or reduction or absence of inhibitory action at intermediate concentrations between the minimum effective and asystolic concentrations. This suggested interaction of two opposing processes.

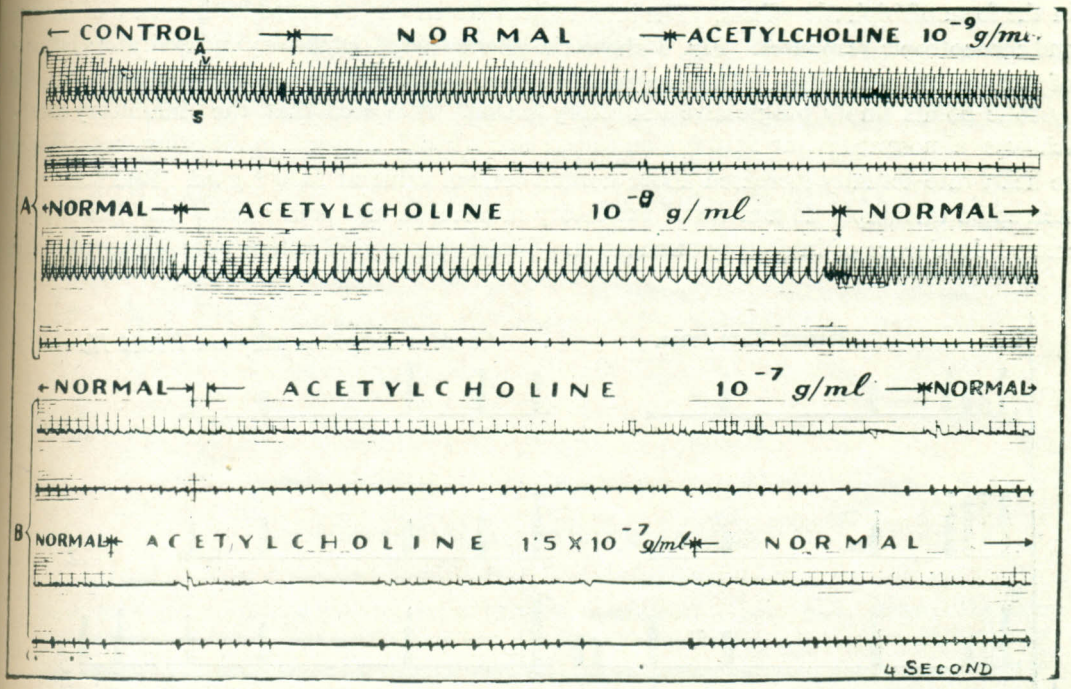

Fig. 1: Extract records of two hearts. Slow speed: Upper trace, ECG. ; lower trace, cardiac output in drops. S sinus, A atrial and V ventricular complexes.

A. Ach 10^{-9} *g/ml* was ineffective while 10^{-8} *g/ml* produced marked effect both on electrical and mechanical activity. Original record run on fast speed showed increased sinoatrial conduction due to which sinus and atrial complexes fused, but a-v conduction was delayed. The auricular and ventricular complexes remained unaltered. The heart rate slowed by 30%. The calculated output was reduced from 2 *ml/min* to 0.6 ml/min.

B. Action at asystolic concentrations. Photographic reduction has masked sino-atrial complexes. At 10^{-7} *g/ml* irregularity of rhythm increased, ventricular extrasystoles occurred and the heart topped. A stronger dose (1.5×10^{-7} *g/ml*) produced three repeated episodes of asystole.

The actions of Ach in 6 hearts illustrating typical and atypical responses are shown in Fig. 2. It would be noted that in heart 1 the inhibitory action of Ach increased typically with concentration till the heart was stopped at 5×10^{-7} *g/ml*. In cane of heart 2 there was a tendency to stabilization of intensity of action between 5×10^{-9} and 10^{-7} *g/ml*. A reduction in the action of Ach is evident in heart 3 at 5×10^{-7} *g/ml*. Clear excitatory action was observed in hearts 4 and 5 where the heart rate and cardiac output increased above normal control values at 5×10^{-7}

and $5x10^{-8}$ *g/ml* respectively. In case of heart 6 the excitatory effects predominated at many concentrations. Stimulation was also suggested by increase in pre-test normal values in many hearts. These unsual concentration-response patterns clearly show variability in the action of Ach. It appears very likely that the stabilization or reduction or absence of inhibitory response is due to interaction of inhibitory and excitatory influences of Ach operating simulataneously. This inference is supported by the occurrence of both excitatory and inhibitory actions separately in the form of biphasic effects. The interaction pattern was observed both in case of inotropic and chronotropic responses. Fig. 3 shows the interaction of inhibitory and excitatory influences of Ach on the pacemaker. The interruped lines connecting mean normal control values with asystolic points imply progressive inhibitory action. It is seen that the inhibitory effect of Ach was almost absent at $5x10^{-8}$ *g/ml* in one heart (Fig 3 A) while in the other heart (Fig.4B) the heart rate actually increased above normal control value at $5x10^{-9}$ *g/ml.* Peaks above the interrupted line imply dominence of excitatory mechanism and points below the line imply increased dominence of inhibitory mechansim. Similar patterns of action were observed in 2 out

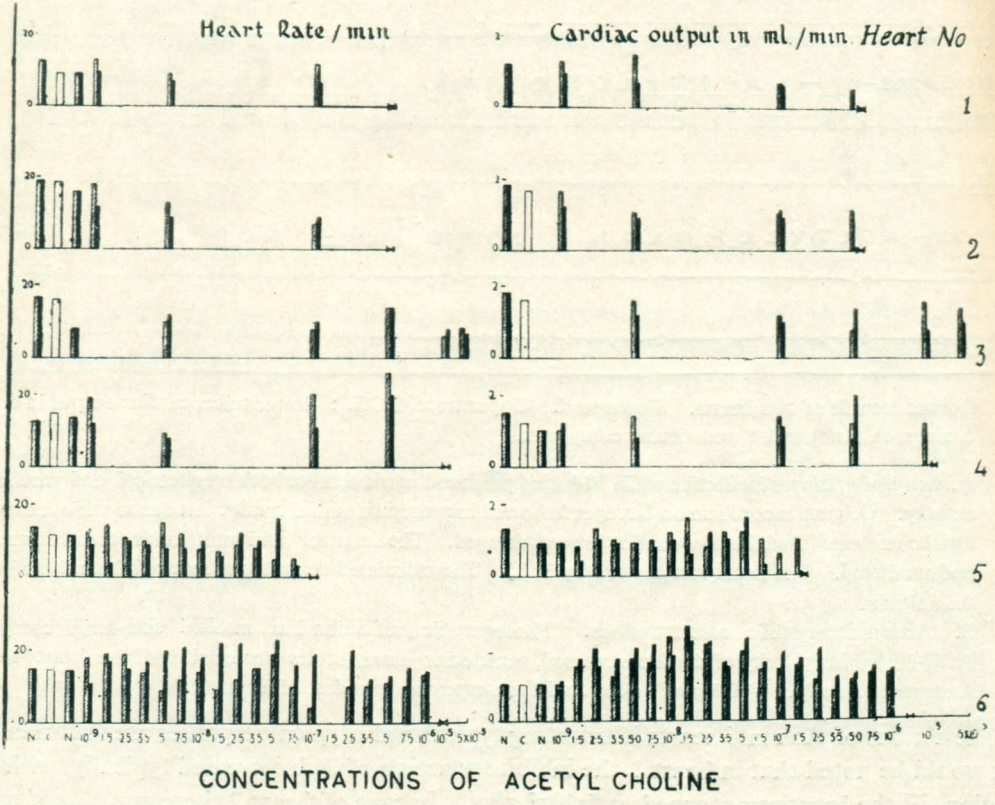

Fig. 2: Action of increasing concentration of Ach on 6 hearts showing various patterns of dose-response relationship. The hatched columns indicate initial normal value while the solid columns indicate final value with each test. The controls are shown by white unhatched columns.

of every 5 hearts both in case of heart rate and cardiac output and were confirmed with fresh solutions by repeated tests. Due to the interaction of two opposing processes the action at a given concentration would be represented by the resultant (or algebraical sum of the two opposing influences.

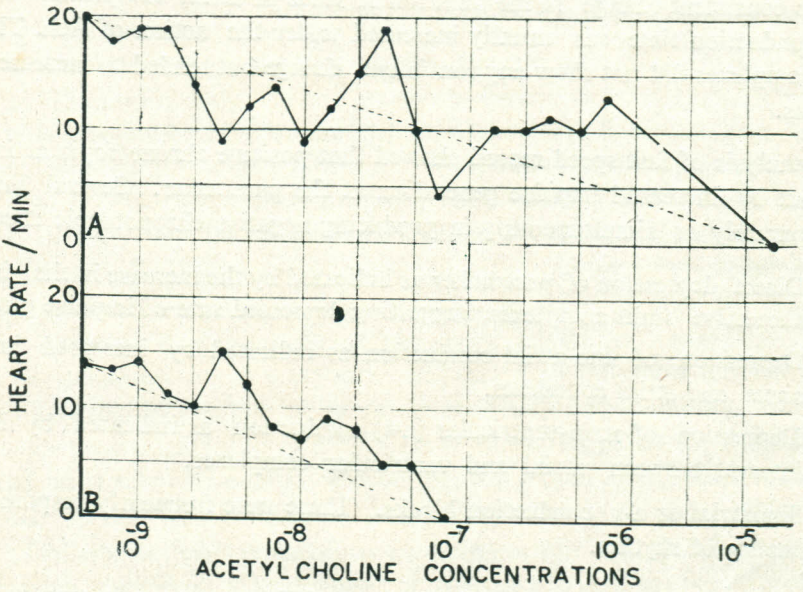

Fig. 3: The chronotropic response of two hearts at different concentration has been shown. The interrupted line connects the mean normal value with the asystolic concentration to indicate progressive inhibitory action. The upper graph (A) shows a sharp peak above the line, indicating a gross reduction in the action of Ach at the intermediate concentration of 5×10^{-8} *g/ml*. The lower graph (B) shows a sharp peak at 5×10^{-9} *g/ml* where the heart rate actually increased above the mean normal control value, suggesting dominent excitatory action.

Electrical Activity: The pattern of ECG recorded from the surface of the heart depended upon the actual placement of electrodes and on the electrical axis of the heart. However the component waves of the cardiac impulse could be easily identified. Each chamber i.e. sinus venosus, atria and ventricle, was represented by a separte wave or electrical complex, Identification of complexes was facilitated by oscilloscopic display and by running the chart paper at a fast speed. Fast speed recording also permitted more accurate measurement of complex duration.

The sinus complexes (S) were inhibited by Ach. The atrial complexes (A) were frequently augmented under the action of Ach at low concentrations but were inhibited at high concentrations. The ventricular complexes (V) also increased at some concentrations in a few hearts but in the majority of hearts there was a reduction in the amplitude of ventricular complexes especially at high concentrations. Thus biphasic effects were also noticeable on the parameters of

electrical impulse of the heart. However the amplitude of ventricular complex did not corre with changes in the cardiac output and was, therefore, not related to the inotropic response

Sinoatrial conduction became faster at low concentrations in some hearts but at h concentrations the conductions was progressively slowed down in the majority of hear There was no consistent change in the duration of sinus or atrial complexes. The atrioven cular (a-v) conduction time was usually increased under the action of Ach. The duration ventricular complexes did not show any consistent change but tended to increase near asysto concentrations.

The analysis of fast speed record showed that positive chronotropic action of Ach w brought about by increased impulse generation at the pacemaker. Several mechanisms wer operative separately or simultaneously in producing negative chronotorpic effect:

(a) Direct depression of pacemaker as indicated by the increase in the interval betwee successive sinus complexes, suggesting depressed rate of impulse generation.

(b) Depression of sino-atrial conduction as indicated by increased interval betwee sinus and atrial complexes.

(c) Depression of atrio-ventricular (a-v) conduction as indicated by increase in t interval between atrial and vantricular complexes.

(d) Sinoatrial or a-v conduction blocks. These were frequently responsible for abno mality of rhythm.

Cernain nonspecific change in ST segment and T waves were frequently observed und the action of Ach and occassionally there was electromechanical decoupling as indicated by pe sistence of electrical activity without the presence of associated mechanical response. High concentration of Ach occassionally produced irregularity of rhythm due to conduction defec and extrasystoles.

The above observations shwoed that Ach acted at three different sites in the hear (i) pacemaker, (ii) conducting system and (iii) cardiac musculature.

DISCUSSION

In the previous study it was observed that the frog hearts are relatively insensitive to Ac during summer specially the months of May and June (2,3,13). The present work further con firms this finding. No heart was found to respond to concentrations smaller than 10^{-9} *g/ml* Also some hearts required 10^{-5} *g/ml* for producing asystole during these summer months in comparison to other periods of the year when almost all hearts are stopped around a concentration of 10^{-7} *g/ml* (13). It is well known that Ach produces both inhibitory as well as excitator (biphasic) effects (6,7,8,9,12). Webb (15) observed post-wash type of stimulation after the withdrawal of dose and following cessation of inhibitory action. Angelakos & Bloomquis (1) have confimed stimulation with nicotinic (high) doses of Ach attributable to catecholamine

release. However other worker, (4,5) have reported stimulation with Ach independent of catecholamine release. The present work was conducted in hot months and the incidence of excitatory effects was more common than observed in winter (13). Thus the incidence of excitatory effects appears to coincide with the relative insensitivity of the inhibitory mechansim of heart to Ach in the summer.

The earlier observation (2,3,13) that th some hearts the action of Ach at intermediate concentrations between the minimum effective and asystolic strengths was less, has also been confirmed by the present study. It has previously been reported (2,3) that if prepared and stored in clean sterile glassware the Ach solutions do not undergo hydrolysis even in alkaline medium and at room temperature for several hours. While analysing the mechanism of variability in the action of Ach at intermediate concentrations, the possibility of hydrolysis was further ruled ou by repeating the tests with freshly prepared soultions. Thus the variability of action in 2 out of every 5 (40 %)hearts was a genuine occurrence and not an artefact. The stabilization of inhibitory action to progressively increasing concentrrtions or reduction or complete absence of inhibitory response at intermediate concentrations represent variants of biphasic action and appears to be due to the intreaction of inhibitory and excitatory influences of Ach. This conclusion is strongly favoured by the present work as excitatory and inhibitory actions were observed separately as well as simultaneously in the form of interaction patterns. The increase in the cardiac output and heart rate above initial normal pre-test values also represents delayed excitatory effects similar to post-wash stimulation observed by Webb (15). Thus under certain conditions the usual inhibitory action is replaced by excitatory action of atypical action due to the interaction of opposing influences. Development of tolerance to Ach has been reported but the reduction or absence of response at intermediate concentrations observed in the present study cannot be regarded as tolerance. In this connection one has to differentiate between reduction of intensity of action of a given concentration or dose and reduction of response to a higher concentration. Further tolerance was not involved in this case because eventually asystole was achieved by higher concentrations, preceded by progressivly increassing inhibitory action. Time factor could also not be implicated as both control and test perfusions were couducted for a fixed period.

It is quite likely that the excitatory and inhibitory influences of Ach are acting through two different-inhibition coupled and excitation coupled-receptors or through two opposite processes. The actual action at a given concentration in a given heart would depend upon the degree of involvement of each type of receptor (or process). and the not effect would be represented by their algebraical sum. Variation in inhibition-coupled or excitation-coupled receptors would also account of the wide variation in sensitivity of the hearts to Ach under different experimental conditions and in different seasons (2).

Acetylcholine produced inhibitory effects on the generation and conduction of impluse as well as on impluse parameters. No difinite correlation can, however, be established between the intensity of mechanical response and the amplitude of ventricular complex. The phenomenon of electromechanical decoupling occassionaly produced by Ach needs further investigation.

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