NATURE OF THE EFFECTS OF ALTERNATING CURRENT SHOCK ON THE SINUS VENOSUS OF THE TOAD

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Strong electrical stimuli were shown by WHALEN (1958) and WHALEN, FISHMAN & ERICKSON (1958) to cause either an augmentation or depression of contractions in the cat papillary or atrial muscle, depending upon the relative amounts of norepinephrine and acetylcholine liberated by the stimulation. These results were confirmed by FURCHGOTT, DE GUBAREFF & GROSSMAN (1959) who indicated further that the release of transmitters does not account for changes in the contraction elicited by threshold stimuli. However, BRADY, ABBOTT & MOMMAERTS (1960) have demonstrated that, in the cat ventricular muscle, a train of supramaximal stimuli produces two actions, a depression of contraction during stimulation and a large potentiation of subsequent contractions. These authors ascribed the former effect to a direct action upon the cell membrane and the latter to a liberation of norepinephrine. Thus, the inotropic effects of strong and weak shocks are quite complicated, although selective stimulation of intra-cardiac postganglionic nerve fibers is possible as shown by LEWARTOWSKI (1963) and VINCENZI & WEST (1963).

On the other hand, the chronotropic effect of electrical stimuli have been demonstrated by WEST (1961), AMORY & WEST (1962), and CORABOEUF, SUEKANE & BRETON (1965) on the mechanical and transmembrane activities of the rabbit heart, and the details of membrane potential change have been studied by LU, LANGE & BROOKS (1965) on the cat sinoatrial node. They found that strong shocks cause cardiac arrest or bradycardia followed by tachycardia with transient hyperpolarization in the nodal cells. Also the hyperpolarization and rate retarding effect are ascribed to a liberation of acetylcholine from the embedded nerve elements, and the secondary tachycardia to that of norepinephrine (see also AMORY & WEST, 1962; VINCENZI & WEST, 1963).

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However, certain aspects of the inotropic and chronotropic effects, particularly differences in effect of direct and indirect actions, still remain to be solved. In this preliminary experiment on the isolated toad sinus venosus, it will be shown that the direct effects of electrical stimulation on the muscle can be divided into two parts; one on the membrane and the other on the intracellular elements. The indirect actions such as inotropic and chronotropic after-effects, on the other hand, are attributable mainly to the cholinergic and adrenergic transmitter actions. The grade and time-course of the effect, however, altered considerably depending on the condition of the preparation and of the stimulation, and three phases of after-potential were elucidated by massive electrical stimulation. The appearance of three phases was noted to relate to a liberation of catecholamines from different storage compartments.

METHODS

The sinus venosus isolated from the toad in the winter months (January to April) was used. The strip of about 2 mm × 7 mm, which was immersed in a lucite chamber containing Ringer's solution, was fixed at one end to the wall and the other end to the sensitive arm of a resistance wire strain gauge. A pair of grid electrodes 10 mm × 40 mm wide were placed with 10 mm interpolar distance parallel to the long axis of the strip in the middle. Thus, massive stimulation with alternating current of 60 cycles was applied to the strip at definite intervals of about 5 minutes, and the effects on the spontaneous rhythm and isometric contractile tension were observed. The voltages used for stimulation were strictly confined within a range of apparent full reversibility.

The Ringer's solution composition in mM was NaCl, 110.35; KCl, 2.50; CaCl₂, 2.15; NaHCO₃, 2.38; glucose, 5.50. The solution was kept flowing continuously in order to minimize the temperature rise due to the stimulating current. The small temperature change which nevertheless resulted was determined by means of an electrothermometer immersed in the solution between the electrodes.

RESULTS

1. *General effects of alternating current on the sinus node.* FIG. 1A illustrates the effects of electrical stimulation of 10 V/cm and 5 sec duration on the mechanical activity. As shown in the figure, the effects could be divided conveniently into an immediate effect (IE) during current flow and an after-effect (AE) after cessation of the current. The after-effect appeared further to be subdivided into an after-inhibition (AI) and an after-augmentation (AA).

FIG. 1B and 1C show the effects of alteration in duration and in intensity of the massive stimulation respectively. As seen in these figures, any increase in duration or strength of the alternating current generally evoked greater depression followed by potentiation. Stimuli only slightly above threshold, however, resulted frequently in potentiation not preceded by depression.

FIG. 2 demonstrates the appearance of three phases of the after-potential in tension when the duration of stimulation was altered. The left figure

FIG. 1. Action of the alternating current on spontaneous contraction of the toad sinus venosus. A) A record showing general effects. S: Electrical stimulation at 10 V/cm and 5 sec duration. IE: Immediate effect during stimulation. AE: After-effect, which consists of after-inhibition (AI) and after-augmentation (AA). The tension change does not appear in parallel with that of rhythm. B) Effects of alteration in duration of the stimuli. (a) 1 sec, (b) 4 sec, (c) 10 sec, (d) 20 sec stimulation at 10 V/cm. C) Effects of alteration in voltage. (a) 4.0 V, (b) 12.5 V, (c) 27.5 V, (d) 40.0 V/cm stimulation of 5 sec duration.

(A) shows a series of time-courses of the peak tension level observed in a normal condition, and the right figure (B) illustrates the actual records obtained from a deteriorated preparation. Note the changes in mode of appearance of the first, second and third potentiation phases (I, II and III) depending on the duration of stimulation. Similar changes in three phases of the potentiation

FIG. 2. Appearance of three phases in the after-potential. A) Time-courses of the peak tension level of rhythmical contractions after electrical stimulation of different durations. B) Actual records in similar conditions but obtained from a deteriorated preparation.

were also observed when the strength of stimulation was altered.

On the other hand, a deceleration followed by an acceleration of the pacemaker rhythm was usually produced by the mass stimulation. Although their time-courses were not analysed in detail, the chronotropic and inotropic effects of the current did not necessarily appear in parallel with each other (see FIG. 6).

2. *Immediate effects of the current flow on the sinus node.* The immediate effects of electrical stimulation when the voltage was less than about 1.7 V/cm were an acceleration of the rhythm and a depression of the contractile tension (FIG. 3). The higher voltages from about 1.7 V to 5.0 V/cm caused an irregularity in the rhythm and contractile tension, probably because of the appearance of fibrillation. Further increase in voltage caused complete depression of the rhythmical contraction and instead the appearance of sustained contraction (FIG. 1C).

FIG. 3A shows the effect of low voltage stimulation on the activity under normal conditions. FIG. 3B and C illustrate the action of acetylcholine (10^{-6} g/ml) and atropine (10^{-7} g/ml) respectively. The voltage range at which fibrillatory contractions appeared was slightly lowered by acetylcholine, while it was raised by atropine. There was also a general enhancement of the after-inhibition by acetylcholine. On the other hand, atropine caused a dramatic abolition of the after-inhibition.

FIG. 4 summarizes the relations between the voltage of stimulation and the immediate effects, in (A) on the tension and in (B) on the rhythm. As seen in these figures, there was a definite voltage range in which fibrillatory contractions appeared. This range was shifted to lower voltage levels by

FIG. 3. Action of acetylcholine and atropine on the effect of long lasting alternating current of weak intensity. A) Control records. B) After addition of acetylcholine (10^{-6} g/ml) and C) atropine (10^{-7} g/ml). Voltage of stimulation is shown in the left side of the records. Duration of stimulation was 20 sec in A and B, and 30 sec in C.

acetylcholine and to higher voltage levels by atropine as noted above (see also FIG. 4B).

In any case, a further increase in stimulation voltage produced a sustained contraction, which persisted and even grew during current flow (FIG's 1C and 5). The peak tension of the sustained contraction increased linearly with further increase in voltage of the stimulation up to 35 V/cm, when it reached about 150% of the maximum tension of the normal rhythmic contraction. This sustained contraction is thought to be produced by direct stimulation of the intracellular elements, since it was produced even after the membrane excitability and twitch contraction were completely eliminated by the action of procaine (10^{-3} g/ml) or by tris-chloride replacement of all external sodium ions. Therefore, the sustained contraction may become a useful indication of the contractility of the cardiac muscle though further studies are necessary.

3. Nature of the after-effects and action of the autonomic transmitters. A series of control experiments showed that the special after-effects of the current on the sinus venosus were almost independent of the temperature rise due to the current flow, but principally dependent on the autonomic transmitter actions.

As already shown, electrical stimulation usually produced either a complete arrest or marked reduction in rhythm of the pace-maker activity despite the increase in heart rate expected from a rise of temperature. FIG. 6 illustrates

FIG. 4. Relation between stimulation at low voltages and the immediate effect. A) The change in isometric twitch tension during current flow. Open circles and continuous line show the mean. B) The change in rhythm. Voltages less than 1.7 V/cm usually produced an acceleration of the rhythm and a depression of the contractile tension. The higher voltages up to 5.0 V/cm caused the appearance of fibrillatory contractions (A). Acetylcholine (10^{-6} g/ml) shifted the fibrillation threshold to a lower voltage level, and atropine (10^{-7} g/ml), to a higher level, as seen in the voltages of peak frequency of the rhythmic contractions (B).

FIG. 5. Dependence of the immediate effect on voltage of the electrical stimulation. A) Voltage-tension relationship. Tension given in percent of normal, 100% being the control value. The lower voltages affected the rhythmical contraction (RC) while the higher voltages elicited a peculiar sustained contraction (SC). Cross ($\times$) shows the highest value and solid circle ($\bullet$) denotes the lowest. B) Some of actual records in case of high voltage stimulation. The lower trace is a response to 19 V, the middle to 27 V and the upper to 36 V/cm, 12 sec stimulation. The stimulation is indicated by an arrow.

a great difference between the actual changes of pace-maker rhythm and the changes predicted from the known temperature dependence of the rhythm. A large difference in contractile tension was also shown between the actual values and the values predicted from the known effect of temperature on contractile tension. Thus, it was concluded that a greater part of the after-effects was not directly related to the temperature change.

On the other hand, acetylcholine (10^{-6} g/ml) and physostigmine (10^{-6} g/ml) both enhanced the first after-inhibition markedly, while atropine (5×10^{-8} g/ml) produced a dramatic disappearance of the inhibition and even caused augmentation (FIG's 7A and B). Similar effects were always observed in spite of considerable changes in duration and intensity of stimulation. Thus, the first inhibitory after-effect of the current appeared mainly due to liberation of acetylcholine by the electrical stimulation.

The second after-effects, acceleration and potentiation were also strengthened by acetylcholine, and the potentiation often appeared in two or three phases. On the other hand, atropine (5×10^{-8} g/ml) and epinephrine (10^{-6} g/ml) suppressed the appearance of both the first inhibition and second augmentation and caused an over-all diminution in effects of the electrical stimulation, probably because of the preliminary and overwhelming augmentation (FIG.'s 7B and C). Isoproterenol (10^{-5} g/ml) produced almost the same results.

Dichloroisoproterenol (10^{-5} g/ml), blocking the β -action of epinephrine, caused a marked depression of the pace-maker rhythm and contractile tension. Under these conditions, no acceleration in rhythm was elicited by electrical

FIG. 6. The difference between the pace-maker activity estimated by its temperature dependence and the actually observed one after the application of alternating current. A) 10.5 V/cm, 5 sec stimulation which is indicated by a solid rectangle. B) 20 V/cm, 5 sec stimulation. In each case, the temperature of the bathing solution after stimulation was shown in a solid line with small dots. The estimated changes in rhythm (broken line) and in contractile tension (chain line) were obtained from the temperature dependence which was determined in other control experiments. The estimated change, however, differed greatly from the change actually observed, not only in the rhythm (solid line with filled circles), but also in the contractile tension (solid line with crosses).

FIG. 7. Effects of acetylcholine, epinephrine and their inhibitors. A) Action of acetylcholine, 10^{-6} g/ml. B) Atropine, 5×10^{-8} g/ml. C) Epinephrine, 10^{-6} g/ml. D) Dichloroisoproterenol, 10^{-5} g/ml. In each figure (a) shows a control, (b) a record about 5 minutes, and (c) 15 minutes after the application of drug. See further explanation in text.

stimulation, but a transient enhancement of the tension occurred (FIG. 7D). However, the second and third potentiation phases were markedly diminished. Similarly, when reserpine (10^{-5} g/ml) was applied after blocking the cholinergic inhibition by pretreatment with atropine (10^{-8} g/ml), the after-acceleration and second and third potentiations were gradually eliminated (FIG. 8A). On the contrary, when cocaine (2×10^{-5} g/ml) was given to the preparation pretreated with atropine, first a marked over-all augmentation of the after-potentiations appeared, but next a depression of the second and then first potentiations were gradually developed to cause a complete arrest of the rhythmic activity (FIG. 8B). This arrest could be no more cured by atropine, but a normal rhythmic activity and an ordinal response to electrical shocks were recovered by reintroduction of Ringer's solution. Thus, adrenergic blocking agents which are known to affect different functional sites appeared to inhibit specifically the different phases of after-potential.

FIG. 8. Action of reserpine and cocaine on the after-effect of the alternating current. The preparations were preliminary treated with atropine (10^{-8} g/ml) and the inhibitory after-effect was eliminated. A) Action of reserpine (10^{-8} g/ml). (a) Control record, and (b) to (f) a series of records 16, 22, 27, 32, and 37 minutes after the application of drug respectively. Note an appearance of three phases in the after-potential and an elimination of the later two phases by reserpine. The tension records of (b) to (f) were obtained at twice the amplification of record (a). B) Action of cocaine (2×10^{-6} g/ml). (a) Record immediate after the application. Note an augmentation of all phases of after-potentials. (b) (c) Records at 10 and 15 minutes after the application. (d) (e) Those at 5 and 32 minutes after washing respectively.

DISCUSSION

Our experiments on the sinus venosus of the toad showed the immediate effect of low voltage alternating current stimulation to be a depression of contraction and a generation of fibrillatory activity, while in the high voltage range the current produced a sustained contraction. The tendency to fibrillation was more apparent in strips of ventricle than in strips of the sinus venosus (unpublished observations) which might explain the rapid appearance of ventricular fibrillation in a whole heart subjected to massive electric shocks of low voltages. The sustained contraction was thought to be a product of direct stimulation of the intracellular elements since it appeared even after the membrane excitability was completely eliminated by the action of procaine or by tris-chloride replacement of all external sodium ions. Though detailed study on the membrane potential change has to be done, the sustained contraction must be specially in view point of E-C coupling in the myocardium.

Among the after-effects of alternating current we have observed an inhibition due to acetylcholine liberation followed by a potentiation consisting of three phases, which were attributable to epinephrine action. The observations coincide well with earlier works of WHALEN (1958), WHALEN, FISHMAN & ERICKSON (1958), AMORY & WEST (1962) and VINCENZI & WEST (1963), on the warmblooded animals though those authors did not discriminate the three phases. Thus it can be concluded that in the sinus venosus of the toad also depression and augmentation of contractility after strong electrical stimuli are mainly determined by the relative amounts of acetylcholine and epinephrine liberated by the stimulation. BRADY, ABBOTT & MOMMAERTS (1960) showed that the potentiation can be divided into two phases; the first rise due to an increase in rate of the tension development and the second due to a prolongation of the active state. Although we have observed similar changes in the time-course of the contraction, the nature of the three potentiation phases in our experiment remains to be clarified.

However, there can be little doubt that at least two storage sites of adrenergic transmitter exist (TRENDELENBURG, 1961; AXELROD et al. 1962; KOPIN et al. 1962; POTTER et al. 1963). WEINER et al. (1962) and AXELROD (1963) have postulated three types of sites or pools. FURCHGOTT et al. (1963) also have studied the actions and interactions of norepinephrine, tyramine and cocaine on strips of thoracic aorta and left atria of the mammals and proposed that there are three types of storage sites; i. e. major but slow compartment (Sm), minor but fast compartment (Sa) and transfer sites (T). If these are also the cases of the amphibian heart, it is highly probable that three potentiation phases in our experiment are some manifestation of the three storage compartments. The elimination of slow major potentiation by reserpine, and

the temporal augmentation of all phases by cocaine and the following depression of fast potentiations will support this view.

SUMMARY

1. The effects of alternating current on pace-maker activity have been studied in the isolated toad sinus venosus. The alternating current produced an immediate action followed by an after-inhibition and an after-augmentation.
2. The immediate action of stimulation by voltages less than 1.7 V/cm was an acceleration of the rhythm and a depression of the contractile tension, while voltages of 1.7-5.0 V/cm caused an irregularity in rhythm and tension. The voltage range in which the irregularity was obtained was lowered by acetylcholine and elevated by atropine.
3. A further increase in stimulation voltage produced, during current flow, a sustained contraction which increased linearly with voltage increase up to 150% of the peak tension of normal phasic contraction. The sustained contraction appeared even after the elimination of membrane excitability by procaine or by complete lack of external sodium ions, and hence it was thought to be a direct response of the intracellular elements.
4. The first after-effect was an inhibition of both pace-maker rhythm and contractile tension, which appeared prominent at higher voltage of stimulation. The inhibitory effect was strengthened by both acetylcholine and physostigmine, and blocked dramatically by atropine.
5. The second after-effect was an acceleration of the pace-maker rhythm and a potentiation of contractile tension. The potentiation appeared frequently separated into three phases, the fast, middle and slow phases. Dichloroisoproterenol and reserpine blocked the acceleration and eliminated the middle and slow potentiations. Cocain, on the other hand, abolished the fast and middle potentiations after a temporal enhancement of all phases.
6. The after-effects of mass stimulation, therefore, are attributable mainly to cholinergic and adrenergic transmitter actions, and it is postulated that three phases of the after-potentiation relate closely to the liberation of adrenergic transmitters from the different storage compartments.

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