THE MODE OF ACTION OF DRUGS ON UNSTRIATED MUSCLE AND THE NATURE OF INHIBITION

By Inderjit Singh, F.A.Sc.

(*From the Department of Physiology, Dow Medical College, Hyderabad, Sind*)

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The action of potassium appears to depend upon a difference in its concentration within and without the fibres, both in unstriated muscle (Singh, 1939) and in striated muscle (Wright, 1942). The mode of action of drugs appears to be different. According to Clark, they attach themselves to sensitive patches on the cell surface, and according to Straub, they act by a virtue of a difference in concentration of the ions on two sides of the muscle membrane, during the process of permeation.

In unstriated muscle, most of the contractions and inhibitions fall into two groups. In this paper, the properties of contractions and inhibitions produced by certain drugs are described, and the mode of their action elucidated.

Results

Action of Acetylcholine

In the electrolyte-free medium :

In this medium the frog stomach becomes hyperexcitable as regards the excitability which produces the spontaneous contractions. The spontaneous contractions may be three times larger in the half tonic sucrose solution than in the saline; this proves that the excitability of the frog stomach is not depressed. The action of acetylcholine in this medium has been very difficult to ascertain. Out of 12 stomachs in six it produced no contraction. This suggests that the contraction produced by acetylcholine is due to sensitisation to ions in the saline, as it is not produced in the absence of such ions, though the excitability of the muscle is otherwise normal. In the remaining six muscles, acetylcholine produced a contraction, but it was smaller than in the saline, and indistinguishable from the sopntaneous contractions; therefore when acetylcholine was added, it was difficult to be certain whether the contraction was due to the drug, or it was one of spontaneous contractions. As it was however a constant phenomenon, I am inclined to believe that it was a contraction produced by the drug.

The effect of the electrolyte-free medium therefore shows that acetylcholine produces two kinds of contractions, one by ions outside the fibres, and the other by ions inside.

Frog stomach.—In this muscle acetylcholine produces two kinds of contractions. One kind of contraction resembles the potassium contraction, in that (i) it is increased if the chloride of the saline is replaced by iodide, which increases the excitability to potassium and ammonium, but depresses that to alternating current. Small concentrations of iodide (0·00560 M NaI) increases the excitability to potassium as well as alternating current by antagonising the inhibitory action of sodium. Greater increase in concentration of iodide, decreases that to alternating current but increases that to potassium owing to its potentiating the action of the latter. If increased beyond 0·056 M, then the excitability to potassium also is depressed; but this action is variable, the concentration which increases the excitability may vary from 0·01 M till the whole of the sodium chloride is replaced by iodide. (ii) It is decreased by increase in osmotic pressure to 1·4 times normal. This contraction is increased by eserine (1 in 10^5). The other contraction has the properties similar to those of contraction produced by alternating current. (1) The optimum concentration of calcium is the same. (2) It is increased by increase in osmotic pressure of the saline to 1·4 times normal at pH 8, sodium chloride being used in increasing the tonicity of the saline. (3) The optimum temperature is the same. (4) It is depressed by iodide. (5) It is decreased by small concentration of eserine (1 in 10^5).

The above results are in agreement with the findings in skeletal muscle, that acetylcholine is capable of causing contracture, as well as twitch. Acetylcholine therefore mobilises potassium within the fibres. From this, it would appear that acetylcholine does produce a contraction in the electrolyte-free medium.

A curious fact was noticed, that the stomach and heart muscle from certain frogs were absolutely refractory to acetylcholine (1 in 10^8 to 1 in 10^4), even after eserine (1 in 10^7 to 1 in 10^4). Like *Mytilus* muscle, they could withstand high concentration of eserine. It is therefore difficult to conceive how acetylcholine could act as transmitter, especially when muscle responded normally to potassium, and alternating current; the latter is known to produce a propagated response. This supports electrical rather than chemical transmission.

Dog stomach.—In the dog stomach the criterion distinguishing the two excitabilities is the concentration of calcium and hydrogen ions.

Here also acetylcholine produces two kinds of contractions. In one kind the optimum concentration of calcium is the same as that for the contraction produced by alternating current (0·002 M $CaCl_2$). In the other, the calcium required varies from 0·007 to 0·03 M $CaCl_2$, so that it belongs to the potassium group. Acetylcholine, therefore, mobilises potassium within the fibres, and increases the sensitivity to ions outside.

Rabbit gut.—In this muscle, acetylcholine produces similar result as in dog stomach.

Guinea pig uterus.—The contraction is favoured by excess of calcium.

Mytilus *muscle.*—Acetylcholine causes a tonic contraction, decreases the excitability to alternating current and increases that to potassium. It differs from the latter, that replacement of the sodium of the saline with lithium decreases the contraction. If excess of potassium has not depressed the excitability, then the contraction produced by acetylcholine is potentiated. This shows two actions of acetylcholine. (1) It produces contraction belonging to the potassium group. (2) Its action depends on the action of ions out side the fibres.

Many *Mytilus* muscles were found absolutely inexcitable to acetylcholine, in fact to chemical stimulation in general. Therefore it is difficult to understand how acetylcholine could be a transmitter of the nervous impulse to the muscle. The muscle responded to alternating current, which produced a propagated response. It is therefore reasonable to suppose that transmission was electrical.

In these muscles, treatment with eserine (1 in 10^6) restored the response not only to acetylcholine, but also to alternating current, potassium. This shows that the action of eserine was a general one on excitability, rather a specific one on any choline esterase. Tone was diminished, when the excitability otherwise was increased; eserine caused relaxation, while acetylcholine caused contraction. Large concentrations of eserine diminished the response to alternating current, but increased that to potassium.

Adrenaline contraction :

Mytilus *muscle.*—The action depends upon the sensitivity of the muscle. In muscles which are sensitive, adrenaline (1 in 10^5) causes contraction, increases the excitability to potassium, stretch, barium; diminishes that to alternating current, and direct current, increases the off-contracture and viscosity, diminishes the rate of relaxation and of stretch. Smaller concentrations increase the excitability to electric current and diminish tone,

In insensitive muscles, adrenaline (1 in 10^5) increases the excitability to electric current, diminishes viscosity and tone. Caffeine (saturated solution in *Mytilus* saline) abolishes the insensitivity. Caffeine in such muscle increases the excitability to alternating current, twitch, potassium.

These results indicate, that the adrenaline contraction belongs to the potassium group, that is produced by the ions outside the fibres.

Frog stomach.—In the frog stomach adrenaline rarely causes a contraction. The contraction is followed by inhibition. The contraction occurs in the electrolyte-free medium, thus showing that adrenaline mobilises potassium inside the fibres.

Such a phenomenon is also produced in the *Mytilus* muscle in the absence of calcium, or if the chloride is replaced by anions such as iodide, in rabbit gut in excess of potassium by alternating current. The initial contraction is followed by inhibition. With diminution of excitability the initial contraction disappears so that inhibition only remains (Fig. 1). As this secondary contraction is adaptation, inhibition and adaptation are therefore identical. Inhibition is therefore a secondary process; it is an excitatory process overcome by adaptation. When a substance produces excitation, adaptation is a concurrent phenomenon, and if the latter proceeds as fast as or faster than the former, then only inhibition would be produced. More intense the excitatory process, the greater will be the accompanying inhibitory process, and with greater concentration of the drug, both the inhibitory and excitatory processes may be increased in such a way that the former predominates. In small concentrations, the drug will produce contraction and with large concentrations inhibition.

It is probably for this reason that many processes affect excitation and inhibition identically, because in the latter, the excitation which is masked, is really the primary process. A substance may thus produce excitation or inhibition. The inhibitory process being the result of excitatory process, rises and subsides more slowly than the latter, with the result that contraction in dog stomach by alternating current, potassium, acetylcholine, vagus stimulation (Narayana and Singh, 1944) is followed by inhibition. If the excitatory and the inhibitory processes balance each other, then on addition of the drug, there will be no significant change, but on withdrawl of the drug, an inhibition would be produced (Singh, 1942).

Another phenomenon frequently met with in unstriated muscle is that in which a substance first produces inhibition, and then contraction. Such action is produced by ammonium, adrenaline, thiocyanate in dog stomach. In this instance, the excitatory process catches up with the inhibitory pro-

cess. On withdrawal, a contraction or inhibition might result. The former will be produced if adaptation takes place to the inhibitory state, by increase in the excitatory state, and the latter when such an adaptation is very slow. Thus on addition of a substance, there may be (1) contraction; (ii) inhibition; (iii) contraction followed by inhibition; (iv) inhibition followed by contraction; (v) no visible effect. On withdrawal, there may be (i) contraction; (ii) inhibition; (iii) or no visible response.

Dog stomach.—Adrenaline may cause contraction or inhibition. The contraction resembles that produced by acetylcholine, in that the concentration of calcium required is about 0·007 to 0·01 M $CaCl_2$. If however adrenaline and acetylcholine are added together, the total tension is not greater, but less than that produced by acetylcholine alone. This is an example of occlusion in unstriated muscle. It is difficult to explain. It means that the excitatory process set up by adrenaline is antagonistic to that produced by acetylcholine. On withdrawal of adrenaline, the excitability to acetylcholine is enhanced, or withdrawal contraction may be produced. Repeated withdrawal of adrenaline may cause the muscle to enter into tonic contraction.

Eserine (1 in 10^5) increases the excitability not only to acetylcholine, but also to adrenaline, and alternating current. This shows that the action of eserine could not be one simply combining with the choline esterase.

Ephedrine (1 in 10^5) may also cause contraction of the dog stomach and potentiate the response to adrenaline, but after the muscle has adapted to ephedrine, the action of adrenaline is antagonised. This shows that the potentiation of adrenaline by ephedrine is due to the summation, so that it acts by other means besides preventing the destruction of adrenaline.

Adrenaline inhibition :

Gunea pig uterus.—Concentration of adrenaline used was 1 in 10^7. Potassium normally present in the saline (0·00616 M KCl), though itself producing marked inhibition (Singh, 1942), antagonises the inhibitory action of adrenaline (Fig. 2 A, B). This is an example of occlusion in unstriated muscle. Excess of calcium or potassium not sufficient to produce a tonic contraction, potentiates the inhibition. Excess of potassium, which produces tonic contraction antagonises the inhibition.

Calcium, normally present in the saline (0·00206 M CaCl) potentiates the adrenaline inhibition. In the absense of calcium adrenaline may fail to produce inhibition, and may even produce contraction (Fig. 2, D). This resembles the action on heart muscle, wherein inhibition is lessened in the

absence of calcium (Gunn, 1944). Excess of calcium favours inhibition. Effect of sodium chloride is variable. If the sodium chloride content of the saline is decreased upto 20% normal, the inhibition by adrenaline increases just as the inhibition by ammonium in the frog stomach. After that the inhibitory effect of adrenaline decreases.

In isotonic solution of sucrose, the muscle passes into a tonic state, and adrenaline fails to have any effect at all. Introduction of calcium and potassium fails to have any effect. Only on the reintroduction of sodium chloride, that the inhibitory effect of adrenaline returns. Sodium chloride is therefore necessary both for certain contractions and inhibitions in unstriated muscle.

In the electrolyte-free medium (Singh, 1944 *a*) adrenaline fails to have any effect, but the introduction of saline produces inhibition, showing that the muscle has not lost the power of inhibition and that external ions are necessary for the action of adrenaline.

The action of hydrogen ions is variable. They potentiate as well as antagonise adrenaline inhibition. If the reaction is changed from acid (pH 6·5) to alkaline (pH 8), the adrenaline inhibition at first increases and then decreases.

Direct current antagonises as well as potentiates adrenaline inhibition. This is probably due to the fact that it produces both kinds of inhibitions. Its antagonistic action is also shown by the fact, that at pH 8, when maximum inhibition has developed by direct current and adrenaline together, the stoppage of the current produces further inhibition (Fig. 2, C). Exactly opposite happens at pH 6·5, because at this pH, the synergistic inhibition is more easily produced by the current.

Ammonium antagonises the adrenaline inhibition, though by itself it may produce inhibition.

The effect of osmotic pressure is most remarkable. Moderate increase in osmotic pressure (1·1 times normal) may increase the inhibitory action of adrenaline (Dale, 1913), but further increase causes a marked decrease; the inhibitory effect of adrenaline may be abolished or converted into an excitatory one.

Acetylcholine antagonises the inhibition.

Optimum temperature for adrenaline inhibition is 30°C; at 37°C, it may be absent.

Rabbit gut.—Potassium antagonises adrenaline inhibition; the effect of calcium and hydrogen ions is uncertain. Increase in osmotic pressure decreases the inhibition.

Frog stomach.—The reactions are similar to those in the guinea pig uterus. Eserine increases the inhibition.

In the electrolyte-free medium, adrenaline loses its power of producing inhibition. The inhibitory action previously referred to (Singh, 1944 *a*) was on the spontaneous contractions by heavy doses (1 in 10^4). If the muscle is thoroughly washed, then adrenaline loses its efiect.

Action of Pilocarpine

This was studied on the rabbit gut and dog stomach. The action was same as that of acetylcholine. Pilocarpine (1 in 10^5) in dog stomach may produce inhibition. Withdrawal of pilocarpine may cause contraction or augmentation of the spontaneous contractions.

Action of Pituitarin

This was studied on the guinea pig uterus. It causes a tonic contraction, which is improved by calcium and decreased by increase in osmotic pressure.

Discussion

Drugs which act in minute concentrations, either produce contractions or inhibitions which are a class by themselves, or they produce contractions belonging to both groups. Certain effects of these drugs suggest that they sensitise the muscle to ions outside (Singh, 1943). Their action is antagonised by increase in osmotic pressure, that is, increase in the concentrations of ions inside the fibres.

The concentration of the drug is too minute *per se* to have any effect on the ionic ratio on the two sides of the muscle membrane. The action must therefore be produced by ions outside the fibres, that are present in significant amount. It is probable that these drugs affect the same structure, acted upon by calcium that is the muscle membrane. They probably produce a change in permeability (Singh, 1944 *b*).

Adrenaline loses its inhibitory effect in the electrolyte-free medium, and in the absence of sodium chloride. This suggests that its action is produced by some constituent of the sodium chloride. Its action is antagonised by potassium, electric current and hydrogen ions. These are the ions which antagonise the action of sodium chloride, again suggesting that adrenaline sensitises the muscle to sodium or chloride.

It is possible that sodium produces contraction or inhibition, depending upon the reaction, and that chloride produce contraction. Adrenaline may produce inhibition by sensitising to sodium and contraction by sensitising

to chloride. When adrenaline and acetylcholine are together (they individually producing contraction, then their action will be synergistic if they sensitise to chloride, and antagonistic if one sensitises to sodium and the other to chloride.

As the action of adrenaline increases in the presence of calcium, it is possible that these drugs act on the muscle membrane by changing the state of calcium. If free calcium in the membrane is suppressed, then a contraction would occur, and if calcium is liberated then inhibition would occur. This view is supported by the fact that though stimulating ions and tone are antagonistic to inhibition, yet a moderate increase favours inhibition. An increase in the excitatory state leads to an increase in the inhibitory state. With moderate increase, the excitatory state just exceeds the inhibitory state, so that on the addition of an inhibitory substance, the balance is upset in favour of inhibition. With great increase, the excitatory state greatly exceeds the inhibitory state, and the combined action of the inhibitory substance and the inhibitory state is less than the excitatory state.

A substance may thus affect inhibition by its inhibitory action *per se*, and by its action on the tone, and these two actions may be antagonistic. Thus the substance may produce different effects in different circumstances. Eserine increases tone; this may result in increase or decrease of adrenaline inhibition.

SIMILARITIES BETWEEN THE RESPONSES OF UNSTRAITED MUSCLE AND THOSE OF NERVOUS SYSTEM—

The responses of the unstriated muscle resemble those of nerve structure, such as the sensory nerve endings and the neurone. Many of the responses which are regarded as peculiar to the nervous system are found in the unstriated muscle.

Properties of the peripheral sense organs and those of unstriated muscle.— In response to a constant stimulus, they respond with a rhythmic succession of nerve impulses. If a constant stimulus in the form of constant current is applied to a nerve fibre, an impulse is set up when the current is made. Plain muscle presents similar phenomenon when stimulated with alternating current; it may respond by (i) continuous tension, (ii) rhythmic contraction, or (iii) only by initial contraction.

Properties of the neurone and unstriated muscle.—In the neurone there are two states, the central excitatory state and the central inhibitory state; the latter outlasts the former. These two states are also found in unstriated muscle, the inhibitory state outlasting the excitatory.

Occlusion.—This is well seen in the unstriated muscle. Thus in the dog stomach, both adrenaline and acetylcholine cause contraction, but when added together the total tension is less than that produced by acetylcholine alone, may be, in fact equal to difference intead of the sum of two.

Subliminal fringe.—In *Mytilus* muscle, both 0·1 M KCl, and replacement of the chloride of the saline by iodide might cause contraction. In insensitive muscle, both may fail to produce a response, but when potassium is added after iodide, a response is obtained. If they both cause contraction in the first instance then their combined action produces greater tension, than the sum of the two when acting singly.

After dischage.—A single stimulus may set up a repetitive discharge. Thus on cessation of alternating current, the muscle may go on contracting rhythmically for some time. In summer *Mytilus* muscles, treated with *Mytilus* saline containing caffeine and adrenaline, there may be rhythmic after-discharge after a single stretch.

Rebound.—The tonic withdrawal contractions are the same phenomenon; it is due to the fact that adaptation takes place to inhibition by increase in the excitatory state.

Recruitment and after-discharge.—Normally the frog stomach when stimulated with alternating current may contract only during the passage of the current, but when cooled, the tension rises more slowly, and continues to rise some time after cessation of the stimulus.

Inhibition may also show recruitment. Thus on stimulating the guinea-pig-uterus with direct current (14 volts), the inhibition may continue after the cessation of the current.

Fractionation.—Normally when the muscle is stimulated with alternating current, it responds with only a fraction of the tension that it is capable of responding under suitable conditions.

Stimulation by hydrogen ions.—The respiratory centre is stimulated by hydrogen ions. Plain muscle may do likewise, but is much less sensitive. Other stimulus may act identically on both. Magnesium depresses tone, as well as nervous activity. Bromides may produce inhibition in the central nervous system as well as plain muscle. Strychnine makes *Mytilus* muscle hyperirritable to potassium.

SUMMARY

(1) Both adrenaline and acetylcholine produce contraction of unstriated muscle in the electrolyte-free medium, suggesting that their action is due to mobilisation of ions within the fibres.

(2) They also produce contractions or inhibitions resembling those produced by ions outside the fibres; this suggests that they may also act by sensitising the muscle to ions outside.

(3) Acetylcholine produces two kinds of contractions in the unstriated muscle.

(4) Inhibition is really an excitatory process, masked by adaptation.

(5) Adrenaline inhibition is antagonised by potassium, ammonium, electric current, hydrogen ions, and increase in osmotic pressure; it is potentiated by calcium, hydrogen ions, and also by electric current. Adrenaline thus produces two kinds of inhibitions.

(6) Eserine acts by means other than combining with choline-esterase.

(7) Ephedrine potentiates adrenaline also by a process of summation.

(8) Drugs produce a contraction, which is a class by itself.

(9) The optimum temperature for adrenaline inhibition is 30°.

References

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Explanation of Plates

Fig. 1. *Mytilus* muscle : The genesis of the inhibition. Stimulation with alternating current for 5 minutes

A. In *Mytilus* saline.

B. Chloride of the saline replaced with bromide.

C. Chloride of the saline replaced with nitrate. Tone is neutralised during adaptation to current.

D. Chloride replaced with iodide. Inhibition occurs during passage of the current. This inhibition is easily seen to be identical with adaptation.

Fig. 2. Guinea pig uterus

A. Inhibition produced by adrenaline (1 in 10^7) in the presence (+K) and absence of potassium (−K).

B. Same as A.

C. Inhibition produced by D.C. (12 volts) and adrenaline from X to Y ; at Y the current is stopped.

D. Effect of calcium on adrenaline inhibition.

E. Effect of temperature on adrenaline inhibition.