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A COMPARATIVE STUDY OF THE EFFECTS OF ETHANOL, ACETYLCHOLINE AND ADRENALINE ON VARIOUS PARAMETERS OF INTESTINAL CONTRACTIONS OF RABBIT

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ABSTRACT

A comparative study was carried out to observe the effects of ethanol, acetylcholine and adrenaline on various parameters of the contraction cycle of rabbit intestine. Acetylcholine and adrenaline were used in concentrations of 10^{-3}, either alone or in combination with absolute ethanol. The results showed that ethanol and acetylcholine had no effect on the rate of intestinal contractions while adrenaline increased it significantly. How-ever, all these drugs decreased the active tension of the contracting intestine, the decrease being minimum for ethanol, moderate for acetylcholine and maximum for adrenaline. On the contrary, a small transient decrease in resting tension was produced by ethanol and this occurred at a very fast rate while adrenaline produced a marked decrease in this parameter, its rate being 6-10 times slower. Ethanol and acetylcholine were also found to increase the resting tension, it being 2 times greater and occurring at a faster rate with acetylcholine. It is suggested that these drugs produce their effect by altering resting membrane potential, Ca^{++} and other ion fluxes.

Introduction

The smooth muscles in the gastrointestinal tract are largely organized as multidirectional overlaid sheets of irregular fibers and are thus responsible for a multidirectional exertion of tension (Huddart, 1975). Since these muscle fibers possess a well defined structure along with a contractile and non-contractile component, the actual volume of the contractile component is of great importance in terms of generating the maximum tension in the fibers (Huddart & Hunt, 1975). Intestine has the inherent property of spontaneous contractions which are regulated by both the parasympathetic and sympathetic nervous systems. This contractile activity of the intestinal smooth muscle is due to spontaneous activity of the pace maker cells which release acetylcholine at the postsynaptic sites (Bumstock et al., 1963). This is always accompanied by the generation of two distinct types of tensions by the intestine, the active tension and the passive tension or tone and both of these are affected by various drugs (Burnstock et al., 1963; Nasreen et al., 1983a, 1983b).

A drug can not produce effect for which there is no structural basis nor create functions that do not pre-exist in the visceral muscles. On the contrary, an experimentally domostrable pharmacological action implies existence of structures whose functions, by being stimulated or inhibited, account for the observed effects (Wilson & Schild, 1968). Thus, acetylcholine stimulates most visceral muscles and increases the force of their con-traction. These actions are characterized by depolarization of the cell membrane, bringing the potential to threshold and initiating action potentials, which in turn elicit a contraction in the muscle. However, Evens et al., (1958) have shown that acetylcholine can still evoke a contraction in the smooth muscle even when the membrane is completely depolarized by isotonic potassium solutions. It is thus suggested that acetylcholine, under such condition, produces its effect by increasing the movements of K^+ and Cl^- in the presence of Ca^{++}. On the other hand, adrenaline inhibits intestinal contraction and decreases the resting tone (Nasreen *et al*, 1988) and this effect is characterized by an inhibition of action potential discharge and a hyperpolarization of the cell membrane. However, Bueding & Bulbring (1964) have suggested that metabolic changes are also concomitant with the inhibitory effects of adrenaline, probably affecting the ion pumps. Regarding ethanol, de-spite the popular belief of its stimulant property, ethanol is entirely depredent in its action on neurons of CNS and its actions are qualitatively similar to those of general anaesthetics (Bergersen and Goth, 1973). Ethanol in concentration of 5-10% blocks conduction in isolated peripheral neurons while in skeletal and cardiac muscles it produces stimulatory effects. In a recent study, Nasreen et al., (1983a) have demonstrated that 2% ethanol produced an initial transient decrease in resting tone of intestine which was followed by an abrupt large increase in it, probably due to a complete depolarization of the muscle membrane. The active tension was also found to be decreased significantly but ethanol was found to have no effect on the rate of intestinal contraction. It has also been suggested that ethanol forms an integral part of water crystal into which it becomes hydrogen bonded and this effect, in the neighbourhood of synapses, may in some unknown way act to block synaptic transmission (Bowman & Rand, 1980).

In most of the pharmacological studies, the investigators generally consider the passive tone to be more sensitive to drug action than the active tension which is basically used for the measurement of rate of contractions. It is however, of utmost importance to also use the time dependent parameters of passive tension while studying the pharmacological effect of drugs as these parameters are equally well sensitive to demonstrate drug induced effects (Nasreen et al., 1985). In the present work, an attempt was therefore, made to measure the time dependent parameters of the passive tension in the rhythmically contracting isolated rabbit intestine as a tool for the observation of the effects of ethanol, acetylcholine and adrenaline in order to visualize some of the basic mechanisms involved in the action of these drugs.

Material and Methods

The isometric contractions were recorded from the freshly dissected intestinal pieces from the region of ileum. These intestinal pieces were fixed vertically in an organ bath according to the method described earlier (Nasreen et al., I983a; 19836). The contractions were recorded on a slow moving paper of physiograph Four-A from Narco Biosystems Inc. Houston, Texas, U.S.A. using a photo electric force transducer Myograph-A also obtained from the same company. The rate of contractions and changes in the active and passive tensions were calculated from the contraction records obtained on a slow moving paper (0.lcm/sec). The methods used for the calculations of active and passive tensions were also the same as mentioned previously (Nasreen et al., 1985). The general experimental setup, the method of dissection and the solutions used have already been de-scribed in detail (Nasreen et al., 1983a).

Results

a) Rate of Contractions:

A comparison of the effect of various drugs on the rate of intestinal contractions is Idemonstrated in Fig. 1. The histograms showed that the normal rate of contractions was not affected significantly by the addition of ethanol and acetylcholine when applied either alone or in the presence of each other. However, adrenaline was found to increase the rate of contractions significantly ($P < 0.0005$) when it was prepared either in normal buffer solution and applied directly to the muscles or when it was prepared in ethanol itself. No such effects were obtained when adrenaline was used on intestinal pieces which had pre-viously contracted in buffer solution containing 1 ml of ethanol.

b) Active Tensions:

The histograms shown in Fig. 2 demonstrated the effects of the above mentioned drugs on the active tensions generated by the intestinal muscles. It was noted that all these drugs decreased the active tension markedly from the normal values, the decreases being highly significant for ethanol ($P < 0.0005$), acetylcholine ($P < 0.005$) and adrenaline ($P < 0.0025$). It was further noted that the decrease in active tension was mini-mum for ethanol, comparatively greater for acetylcholine and maximum for adrenaline when the last two drugs were applied in the presence of ethanol. Similarly, the decrease of active tensions by various acetylcholine solutions was found to be significant ($P < 0.025$) when compared with ethanol. No such results were obtained for any of the adrenaline solutions and all the decreases in active tensions were non-significant ($P <0.05$)

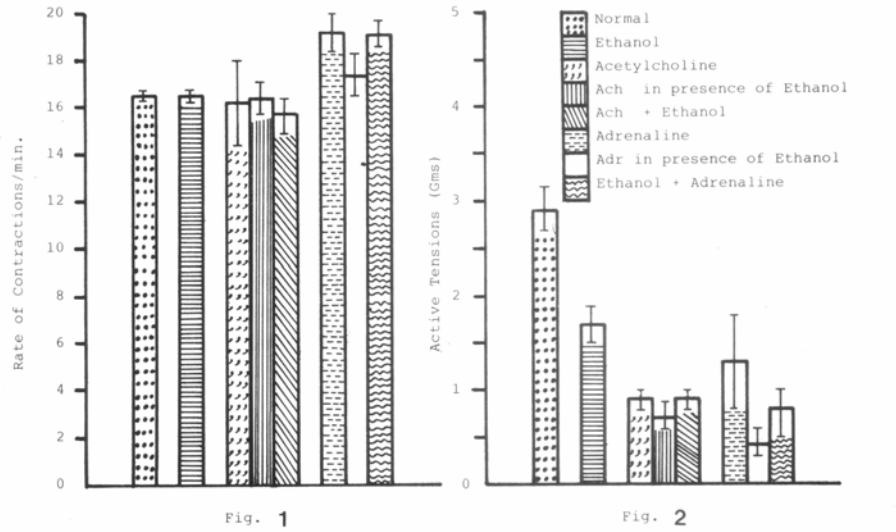

Fig. 1 & 2: Effect of ethanol. accetylcholine and adrenaline m the rate of contractions and active tensions of rabbit intestines.

c) *Decrease in Resting Tensions:*

In the present experiments, a decrease in resting tensions was observed only after the addition of either ethanol or different adrenaline solutions (Fig. 3). No such effects were produced by any of the acetylcholine solutions. Generally, the decrease of resting tension by ethanol was very small and transient. A marked decrease in resting tensions was always produced by adrenaline solutions. A comparison showed that the decreases in resting tensions by adrenaline, (either in buffer solution or in ethanol) was significantly greater when compared with the same effects produced by ethanol.

d) *Time of Maximum Decrease in Resting Tensions:*

Attempt was also made to determine the time period required for the maximum decrease to occur in resting tensions. These results are shown in Fig. 4. It was observed that the small transient decreases in resting tensions occurred at a very fast rate after ethanol administration. Similar results were also obtained in all the three solutions of adrenaline where this drug produced a significant decrease in resting tensions which attained a maximum value in approximately 6-10 times longer time periods. Acetycholine did not produce a decrease in resting tensions in any of the above described experiments.

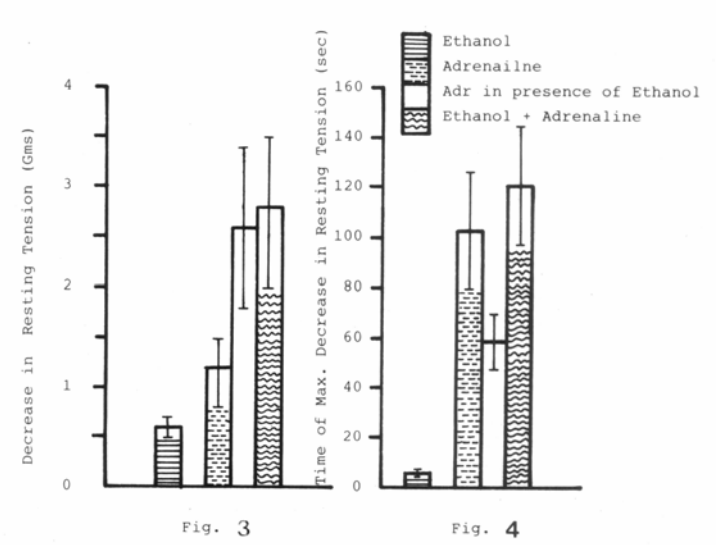

Fig. 3 & 4: Effect of ethanol and adrenaline on the resting tensions and the time of resting tension of rabbit intestine.

e) *Increase in Resting Tensions:*

An increase in resting tensions was generally observed after the application of either ethanol or acetylcholine solutions. The resting tensions showed lesser increase after the addition of ethanol but these were very high after acetylcholine administration (Fig. 5). A comparison showed that the increases in the resting tensions produced by acetylcholine were 2 times greater than those observed in ethanol when the drug was prepared either in buffer solution or in ethanol. In the present experiments, the maximum increase in resting tensions was observed when acetylcholine was prepared in ethanol, the increase being significantly greater than those observed for other acetylcholine solutions ($P<0.005$).

f) *Time of Maximum Increase in Resting Tensions:*

In all the experiments using acetylcholine solutions, the increase in resting tensions occurred at a comparatively faster rate than that of ethanol (Fig. 6). The time periods were that therefore, smaller for the first two acetylcholine solutions and these were significantly smaller than those observed in ethanol ($P < 0.005$). These results suggested that both the ethanol and acetylcholine solutions were capable of increasing the resting tensions. However, the effect produced by ethanol was not only smaller but also occurred at a slower rate. On the other hand, the increase of resting tensions by acetylcholine solutions was much higher and occurred at a faster rate.

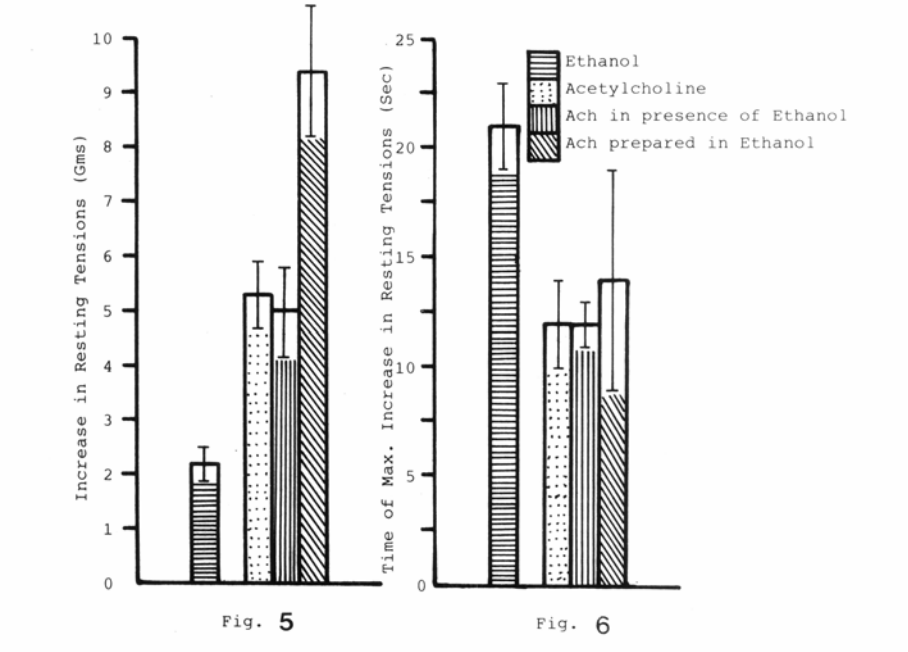

Fig. 5 & 6: Effect of ethanol and acetylcholine on the resting tensions and the time of maximum increase in resting tensions of rabbit intestine.

Discussion

The intestinal smooth muscles come in the category of single-unit rhythmic type where Auerbach plexus acts as a pacemaker to initiate the rhythmic contractions. The rhythmic contractions are tonic in nature and are known as tonic contractions. The extent of these tonic contractions depends upon the extent of muscle activation (Bohr and Uchida, 1969). Bulbring (1955) has demonstrated that most of the smooth muscles are stimulated by moderate stretch which causes membrane depolarization and initiation of spikes. In our experiments, the initial resting tension or passive stretch resistance was fixed at 2 gms for about 1 inch long intestinal pieces. This 2 gm resting tension gave maximum contraction amplitude. Our results demonstrated no change in the rate of con-tractions after the addition of ethanol. This indicated that the pacemaker; cells located in the Auerbach plexus were not affected by ethanol and that ethanol acted directly on the muscle fiber membrane. The active tension or the amplitude of contractions were how-ever, significantly decreased by the action of ethanol. These results were not un-expected since the length tension relationship of intestinal smooth muscles has been shown to be similar to those of skeletal muscles where the increase of passive tension is a continuously rising function of muscle length, but

active tension is not. The reason for this is that as the muscle is progressively stretched, the rise in passive tension is so great that it may eventually exceed active tension (Csapo, 1960). Under such conditions, although the total tension rises, the active tension falls as was observed in the present experiments. Ethanol produced two major effects on the contracting intestine which included an initial transient decrease in resting tension which was followed by an abrupt sharp increase in resting tension, superimposed by small peristaltic contractions or active tensions. This could be regarded as a diphasic action of ethanol. The initial transient decrease could be related to a hyperpolarization of the intestinal smooth muscles. We have neither measured resting membrane potentials nor ionic fluxes in our experiments. However, if it is assumed that intestinal smooth muscles of rabbit behaved like those of toad and dog stomach, than a hyperpolarization of the cell membrane due to increased K^+ efflux would be responsible for a decrease in resting tension. Regarding the stimulatory effect of ethanol or an in-crease in resting tension, it is probable that ethanol in our experiments must have selectively increased Na^+ influx to depolarize the membrane which in turn resulted in an abrupt increase in resting tension. It is however, very unlikely that ethanol may have affected the acetylcholine receptors at the neuromuscular junctions to increase Na^+ permeability since they am specific for the neuro-transmitter only. There may be some other possible sites of action for ethanol to react upon to mobilize Na^+ to the inside of the cell. Ac-cording to Breeman et al., (1979), there *are* anionic sites located on the external or internal surfaces of the cell membrane which bind Nat It is probable that in our experiments ethanol caused the movement of this bound Na^+ to penetrate the sarcolemma.

In our experiments, the ethanol produced increase in resting tension could be easily divided into two components, i.e. an initial fast and a later slow component. In the initial fast component, the resting tension increased at a very fast rate and about 99% of the tension developed within 4-18 sec., with an average time period of 8.5 sec. These observations competed us to believe that ethanol activated Na^+ entry into the cell, causing depolarization and associated increase in free ionic Ca^{++}, occurred at a very fast rate. These observations were also found to be in accordance with the results obtained by Bulbring and Kuriyama (1963), who noted that acetylcholine 10^{-6} increased resting tension in guinea pig taenia coli within 4-20 sec. after the addition of the drug.

In our experiments, acetylcholine (Ach) 10^{-3} was used and in most of the experiments, this higher concentration of acetylcholine produced a complete contructure which, in some cases, was superimposed by small contraction waves. It is generally accepted that acetylcholine increases the excitability of smooth muscles and usually causes con-tractions either by enhancing rhythmic activity in single-unit muscle fiber, or by initiation of activity in multiunit non-rhythmic muscles (Bumstock et al., 1963). Since smooth muscle cells possess specific acetylcholine receptors at the neuromuscular junction, it is postulated by

Burnstock (1958) that Ach acts on smooth muscle cells by simultaneously increasing the permeability of Na^+, K^+ and probably of some other free ions present in the same way it acts at the skeletal muscle motor endplate (Castillo and Katz, 1954). If it is assumed that Ach produced similar depolarization in our experiments, this would be expected to be associated with an increase in free ionic Ca^{++} in the sarcoplasm. Thus, a higher level of Ca^{++} in the sercoplasm would account for a large increase in resting tension as observed in our experiments and this would be regarded as myogenic non-tetanic tone, since it was not dependent on nervous stimulation and occurred spontaneously after the application of Ach. We believe that Ach acted upon both the preganglionic and post ganglionic sites to produce a sustained depolarization of the muscle membrane. We also believe that Ach-produced depolarization kept the membrane potential below the reversal potential level since many of our experiments showed contraction waves superimposed on the sustained contractions. Since acetylcholine-dependent increase in resting tension was about double of that produced by ethanol in our experiments, it is concluded that membrane depolarization and Ca^{++} release mechanisms are more effective when they are generated at the receptor sites. The time measurements further indicated that acetylcholine-produced maximum increase in resting tension occurred in about 12.4 sec. as compared to 21 sec. measured for ethanol. These results also support our belief that receptor dependent depolarizations and release of free ionic Ca^{++} occur at a comparatively faster rate and produce larger tensions.

Acetylcholine produced depolarizations have also been shown to be associated with an increase in spike frequency in spontaneously active tissues, e.g. taenia coil (Bulbring, 1954; 1955; 1957), and rat uterus (Marshall, 1959). We therefore, believe that acetylcholine also produced a train of spikes in our experimental smooth muscles with there being no change in the rate of contractions as has been suggested earlier (Huddart and Hunt, 1975). Since acetylcholine produced larger tension than ethanol in our experiments, we believe that acetylcholine, in addition to its other effects, also produced a synchronization in the spread of depolarization waves through the nexuses (Csapo, 1960). Similar effects have also been suggested for other drugs as well (Burnstock et al., 1963).

In those experiments where acetylcholine was applied to intestinal pieces which had previously contracted in presence of ethanol, the results obtained were almost exactly the same as those observed for acetylcholine alone. In these experiments, both ethanol and acetylcholine produced their individual effects one after the other, with the acetylcholine effects seen superimposed on the effects of ethanol. These results clearly suggested that ethanol did not alter the mechanism of action of acetylcholine, indicating that both the drugs had separate sites of action as discussed earlier. However, the application of ethanol-prepared acetylcholine had some marked effects on the contracting intestine. The average

maximum increase in resting tension was observed to be 9.35 ± 1.17 gms. which was about 33% greater than the sum of the individual effects of ethanol and acetylcholine. It is to be pointed out here that, in our experiments, both the ethanol and acetylcholine produced the same effect, i.e. an increase in resting tension. Thus, it was expected that a simultaneous application of the two drugs would produce greater effects as observed in our experiments.

The time measurements for the maximum increase in resting tension indicated that 9.35 gms. tension was developed in an average time period of 14.3 sec. A look at these results gives the idea as though ethanol-prepared acetylcholine is taking more time to develop tension. However, calculations of the rate of tension development per second indicated that the resting tension increased by 0.25 gms/sec. in ethanol, 0.42 gms./sec. tylcholine and 0.65 guts /sec. in ethanol-prepared acetylcholine. These values clearly indicated that ethanol-prepared acetylcholine not only developed maximum increase in resting tension but it also occurred at the fastest rate. The possible involvement of membrane depolarizations and associated Ca^{++} release, in the generation of above mentioned effects, has already been discussed in detail. Our results further demonstrated that ethanol-produced increase in resting tension started decreasing (Fig. 7) after reaching a maxi-mum value within a very short time period. This effect could be explained on the assumption that ethanol-produced membrane depolarizations were later followed by a repolarization due to K^+ loss, an effect which was also observed for histamine by Born and Bulbring (1956). We suspect that this K^+ loss was blocked by larger depolarization of the membrane due to acetylcholine action. In other words, it can be stated that acetylcholine potentiated the effect of ethanol, provided the two drugs were applied simultaneously. This blockage of K^+ efflux by acetylcholine would result in greater depolarization of the membrane for a longer time period, resulting in a larger tension development. This would account for the 33% greater tension development when ethanol-prepared acetylcholine was used.

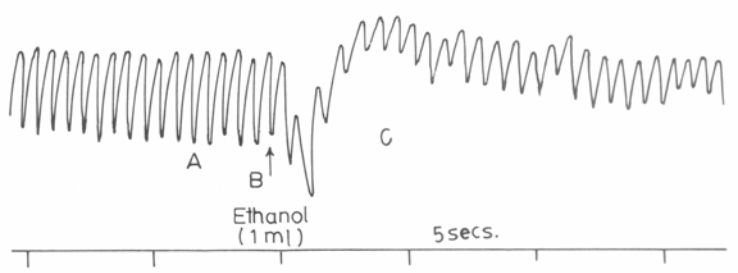

Fig. 7: Effect of ethanol on the active and passive tensions of rabbit intestine. Arrow indicates the Addition of 1 ml. Absolute Ethanol. A= Initial Resting Tension level, B = Transient Decrease in Resting Tension, C=Increase in Resting Tension.

Our results further demonstrated that ethanol-prepared acetylcholine decreased active or tonic tensions by about 3 times while the total tension increased to a very high value of about 10.6 gms. Bulbring (1957) has demonstrated that the changes in spike frequency undergo due to changes in configuration. We believe that similar change in spike configuration has occurred in our experiments.

We have again used high doses of adrenaline (10^{-3}) in our experiments on the intestine. The results showed that the application of high doses of adrenaline produced a more or less complete relaxation and an inhibition of contractions in the intestine. In some experiments however, very small contraction waves were observed. In those preparations however, where adrenaline causes relaxation and loss of tone, as has been observed in our experiments, this effect is associated with a repolarization of smooth muscle membrane and a reduction or blockage of spike activity (Bulbring, 1954; 1957). This adrenaline-produced inhibition can again be explained in terms of electrophysiological phenomena. Since intestinal muscle cells have been shown to be innervated by sympathetic nervous system, there is evidence that *they* possess specific receptors α and β_2 for adrenaline (Huddart and Hunt, 1975). There is also evidence that the inhibitory effect mediated by α receptors is through an action on the neural elements in Auerbach plexus and that adrenaline may directly inhibit some muscle fibres through (i_i receptors in the fibre membrane. We also believe that adrenaline relaxed intestinal pieces in our experiments by producing a repolarization of the membrane by K^+ efflux which is probably followed by an active Na^+ extrusion with a simultaneous large uptake of K^+ and a hyperpolarization of the membrane, resulting in relaxation of the muscles. As we have not observed a decrease in the rate of contractions but actually a slight non-significant increase in it, it is tempting to speculate that the degree of adrenaline-produced after-hyperpolarizations were not great enough in our experiments to delay the individual spikes and thus no change occurred in the rate of contractions. Similarly, an explanation for the observed decrease in active tension may be that adrenaline may have abolished a train of spikes that may have been generated before drug application, a phenomena just opposite to that discussed earlier for acetylcholine.

Our experiments showed that the decrease in resting tension, resulting due to relaxation of the muscles, was on the average 1.19 ± 0.33 gms. It is to be remembered that the intestinal pieces, in all our experiments, were fixed at an initial resting tension of about 2 gms. This would mean that after relaxation, the muscles had a tension of about 0.8 gms. only. According to the length tension measurements for visceral muscles (Csapo, 1969), such a small resting tension by itself will reduce or abolish the motility of the intestine even if all the other factors were excluded. This would also explain the 54% decrease in active tension as a result of adrenaline action in our experiments. Our results also

demonstrated that the major decrease in resting tension occurred at a very fast rate, within an average time period of about 11.28 sec., after the application of adrenaline. This faster action of adrenaline would be possible only if it is assumed that the drug produced its effect through its α and β_2, receptors. Our time measurements of the slow component (resulting due to adrenaline action) seems to be of minor importance since a very small decrease in tension occurred in a very large time period of about 92 sec. This small decrease in resting tension may probably be the result of a firm binding of intracellular Ca^{++} during prolonged action of adrenaline.

In those experiments where adrenaline was applied to intestinal pieces which had previously contracted in presence of ethanol, adrenaline effects on both the active and passive tensions were found to be potentiated. It was observed that all the effects produced by adrenaline were superimposed on the effects of ethanol. The resting tension was found to be decreased on the average by 2.59 ± 0.82 gms. This large decrease in resting tension seems to be misleading since the initial resting tension, at which the intestinal pieces were fixed, was only about 2 gms. This discrepancy in resting tension values was due to two reasons. Firstly, the resting tension varied from experiment to experiment at the time of adrenaline application since ethanol had previously been applied. In general, it was slightly greater than the initial value of 2 gms. Secondly, in many of the experiments, the initial resting tension of the intestinal pieces was slightly increased so that the degree of relaxation could be recorded to a maximum level. In any case, the adrenaline-produced decrease in resting tension was potentiated in presence *of* ethanol. Since ethanol is believed to have increased the resting tension by depolarizing the membranes (see earlier discussion) and as adrenaline has been shown to relax the muscles by hyperpolarization (Bulbring, 1954; 1957), it could be assumed that adrenaline-produced hyperpolarizations were directly related to the degree of earlier membrane depolarizations.

In those cases where ethanol-prepared adrenaline was used, the situation was some what complicated. It is to be remembered that ethanol and adrenaline produced opposite effects in our experiments, i.e. ethanol increased resting tension while adrenaline de-creased it. It would therefore, be expected that on the application of ethanol-prepared adrenaline, both the drugs had an equal chance to produce their opposite effects almost simultaneously and that the recorded effects would be the net difference of these two opposite effects. Our results showed that the decrease in resting tension was about 2.75 ± 0.78 gms., which was slightly greater than the same effects obtained either with adrenaline alone or when adrenaline was applied in presence of ethanol. This decrease in resting tension occurred within an average time period of 14.85 sat. The calculations showed that the resting tension decreased at a very fast rate of 0.19 gms/sec. This value was greater than the same values obtained for other conditions of adrenaline administration (0.105 gms/sec. for adrenaline alone and 0.115 gms/sec for

adrenaline in presence of ethanol). These results clearly demonstrated that adrenaline completely blocked the mechanism by which ethanol increased the resting tension and this probably occurred by its faster action on the muscle cell membrane via its receptors.

The most remarkable effect observed in experiments using ethanol-prepared adrenaline was a significant increase in the rate of contractions. It is very tempting for us to speculate that in our experiments, adrenaline produced hyperpolarizations leading to a very large decrease in resting tension and that these hyperpolarizations were simultaneously superimposed by a large number of spike potentials. It is probable that ethanol may have been responsible for this increased spike frequency. Since an increase in spike frequency is associated with a faster rate of repolarization, a simultaneous shortening in spike duration and a merging of individual after hyperpolarization phases, leads to a greater muscular relaxation.

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