# EFFECT OF ELECTROLYTES ON THE SPONTANEOUS RHYTHMICITY OF RABBIT-DUODENUM

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The role of extracellular $Na^+$, $K^+$ and $Ca^{++}$ on the isolated duodenal contractions has been studied in 40 rabbits. A series of modified Ringer-Locke solutions were used in which the various electrolytes i.e. NaCl, KCl and $CaCl_2$ were replaced by osmo-equivalent amounts of sucrose to maintain their isotonicity. The effect was observed on the rate, rhythm, tone and amplitude (force) of duodenal contractions.

Lowering of NaCl concentrations from 0.90% to 0.60% did not show any change. On further lowering the concentration, the duodenal contractions showed diminution in rate, amplitude and tone; while below 0.30%, the rhythm became irregular and later the muscle lost spontaneous rhythmicity. On lowering the concentrations of KCl, there was diminution in rate and amplitude, which was much less as compared with NaCl. In the complete absence of KCl, the rhythm became irregular, while the tone showed a rise which was not maintained. Diminution of $CaCl_2$ concentration caused reduction in rate, amplitude and tone while in its complete absence the contractions first became irregular and then stopped completely. This indicates that concentrations of NaCl in Ringer-Locke solution above 0.60% is not necessary for duodenal rhythmicity. All the three cations are necessary for normal excitation, though the role of $K^+$ is relatively less important.

Literature is rich in studies pertaining to the variations in the ionic environment of nerve and skeletal muscle which has contributed much to our knowledge of ionic basis of their motility (Hodgkin, 1947, 51; Hodgkin and Katz, 1949; Hodgkin and Huxley, 1950, 52). Although comparatively much work has been done on smooth muscle of stomach (Singh and Rao, 1940; Singh and Acharya, 1957; Bozler, 1960); small intestines of rat ( Clarkson and Rothstein, 1960 ) etc., yet quite little information is available regarding the effect of different ions on the motility of rabbit duodenum. It is known that differences exist not only between the various smooth muscles of the same species but also between anatomically and physiologically comparable smooth muscles of related species. The present investigation was, therefore, undertaken to study the relationship between the external concentration of important cations i.e. $Na^+$, $K^+$ and $Ca^{++}$ and the activity of the isolated duodenum of rabbit.

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## METHODS

Adult healthy rabbits of either sex were used for the experiments. The animal was starved for about 12 hours and only water was given. The animal was killed by stunning and 3 to 5 cm. piece of duodenum was removed and its contractions were recorded in the thermostatic Dale's isolated organ bath. The oxygen was bubbled at the rate of 30-40 bubbles/min. and the temperature of the organ bath was maintained at 37°C.

Contractions were recorded on the slow moving Brodie's Electric Kymograph and the time tracing was taken at 5 secs. interval.

Ringer-Locke solution was used as the basic perfusion fluid. The modified Ringer-Locke solutions containing various concentrations of NaCl, KCl and $CaCl_2$ were prepared by replacing different quantities of NaCl, KCl and $CaCl_2$ by osmoequivalent sucrose. In the preliminary experiments the concentration of NaCl was progressively changed from 0.90% to 0.00% in steps of 0.1% both in the descending and ascending series. As no marked effect was observed on lowering the NaCl concentration up to 0.60%, detailed study of the solutions from 0.60% to 0.00% (NaCl-free Ringer-Locke solution) was done. The duodenum was first kept in Ringer-Locke solution for some time. When it showed normal rhythmic contractions, the solution was replaced by modified Ringer-Locke solution of specific concentration and its effect was studied for one hour. This was replaced by Ringer-Locke solution and the effect was observed for half an hour to find out whether recovery was complete or any irreparable change occurred to the muscle. The same technique was followed to study the effect of $K^+$ and $Ca^{++}$.

The temperature of different solutions was brought to 37°C before transferring them to the organ bath to minimise the effect of temperature.

## RESULTS

The whole work has been divided into 3 sets of experiments :—

1. *Effect of Sodium* :—On changing from 0.90%to 0.60% (modified Ringer-Locke solution) almost no change in spontaneous rhythmicity of muscle was observed. But on further lowering the concentration of NaCl, there was definite diminution in rate, tone and amplitude of duodenal contractions. The diminution was greater with lower concentrations. In NaCl-free modified Ringer-Locke solution, the rhythm became irregular and ultimately the muscle lost spontaneous rhythmicity.

No irreversible change was produced in the muscle in low NaCl concentration up to 0.10%. In NaCl-free Ringer-Locke solution, some muscles showed incomplete recovery, possibly due to certain irreversible changes in the muscle fibres.

The effects of lowered concentration of $Na^+$ are depicted in Table I.

TABLE I

*Effect of Sodium on Rate, Rhythm, Tone and Amplitude of Spontaneous Duodenal Rhythmicity*

| S. No. | Concentration of NaCl | Percentage change in Rate | Tone | Amplitude | Change in Rhythm |
|---|---|---|---|---|---|
| 1 | From 0.80% to 0.60% | Nil | Nil | Nil | Nil |
| 2 | 0.50% | - 2.9 | - 10.9 | - 20.6 | Regular |
| 3 | 0.40% | - 7.4 | - 29.4 | - 72.5 | Regular |
| 4 | 0.30% | - 7.5 | - 20.9 | - 80.0 | Regular |
| 5 | 0.20% | - 83.0 | - 20.3 | - 96.0 | Regular/Irregular/Stopped |
| 6 | 0.10% | - 88.1 | - 16.4 | - 98.6 | Irregular/Stopped |
| 7 | 0.00% | - 96.9 | - 16.3 | - 98.9 | Irregular/Stopped |

2. *Effect of Potassium*:—There was progressive decrease in rate and amplitude of contraction on lowering the concentration of $K^+$ from 0.042% to 0.00% (KCl-free modified Ringer-Locke solution). In $K^+$-free modified Ringer-Locke solution though the rhythm became irregular, yet the muscle did not lose its rhythmicity.

On lowering the concentration of $K^+$ from 0.042% to 0.012% or 0.00% there was initial rise in tone which was not maintained and was followed by diminution in tone.

The recovery in rate, rhythm, tone and amplitude was complete in most of the cases; but in some the recovery in tone and amplitude was incomplete indicating that low concentration of $K^+$ does cause some irreversible change in the muscle.

"K-paradox of Libbrecht" observed in cardiac muscle was also observed in duodenal muscle. When $K^+$-free Ringer-Locke solution was changed to normal Ringer-Locke solution, the duodenum lost its rhythmicity which returned after 10 minutes.

The effects of lowered concentration of $K^+$ are summarised in Table II.

TABLE II

*Effect of Potassium on Rate, Rhythm, Tone and Amplitude of Spontaneous Duodenal Rhythmicity*

| S.No. | Concentration of KCl | Percentage change in Rate | Tone | Amplitude | Change in Rhythm |
|---|---|---|---|---|---|
| 1 | 0.032% | —1.6 | +10.7 | — 9.3 | Regular |
| 2 | 0.022% | —1.7 | + 8.4 | —10.6 | Regular |
| 3 | 0.012% | —2.7 | + 1.4 | —23.7 | Regular |
| 4 | 0.000% | —6.9 | —10.3 | —74.0 | Regular/Irregular |

*3. Effect of Calcium*:—As $Ca^{++}$ concentration was reduced in the Ringer-Locke's solution, there was diminution in rate, rhythm, tone and amplitude of contraction. In $Ca^{++}$ free modified Ringer-Locke solution, the rhythm became irregular and ultimately the duodenum lost its spontaneous rhythmicity.

When modified Ringer-Locke solution was changed to normal Ringer-Locke solution, there was complete recovery in rate, rhythm, tone and amplitude in most of the cases; while in some the amplitude showed incomplete recovery.

The effects of lowered concentration of $Ca^{++}$ are shown in Table III.

TABLE III

*Effect of Calcium on Rate, Rhythm, Tone and Amplitude of Spontaneous Duodenal Rhythmicity*

| S.No. | Concentration of $CaCl_2$ | Percentage change in Rate | Tone | Amplitude | Change in Rhythm |
|---|---|---|---|---|---|
| 1 | 0.014% | — 6.3 | — 0.1 | +13.7 | Regular |
| 2 | 0.004% | —12.7 | —18.4 | —86.4 | Regular |
| 3 | 0.000% | —85.2 | —35.7 | —98.7 | Irregular/Stopped |

## DISCUSSION

From the observation that the spontaneous rhythmicity of frog's striated muscle, which was lost in isotonic sucrose solution, was restored by even 0.07% NaCl solution and to a lesser extent by Lithium salt, Overton (1902) suggested that $Na^+$ is essential for excitability and conductivity of muscle. These findings were later confirmed by Urano and Fahr (1908). While working on the giant axon of squid,

Hodgkin and Huxley (1947, 1950, 1952) and Hodgkin and Katz (1949) have put forward the "Sodium Hypothesis" to explain the ionic basis of bio-electric potentials. Fleckenstein (1957) has remarked that the energy available from the inflow of $Na^+$ and outflow of $K^+$ across the fibre membrane during excitation is sufficient to account for the work of contraction.

The present study has shown that $Na^+$ ( up to 0.60% NaCl ) is essential for spontaneous rhythmicity of rabbit duodenum and the muscle loses this rhythmicity in NaCl-free modified Ringer-Locke solution. In some experiments, the muscle has remained excitable for some time in NaCl-free modified Ringer-Locke solution. This can be explained by the fact that though the solution is NaCl-free, yet it is not $Na^+$-free as Ringer-Locke solution contains 0.050% $NaHCO_3$. The $Na^+$ is also essential for contractility as diminution in NaCl causes diminution in amplitude and tone.

As no effect has been observed on lowering the concentration of NaCl to 0.60%, one is inclined to believe that excess of NaCl ( more than 0.60% ) used in classical Ringer-Locke solution is not necessary for normal excitability of the muscle but has possibly the function of maintaining osmotic pressure. Winton and Bayliss (1955) also remarked that the amount of $Na^+$ present in the Ringer-Locke solution was greatly in excess of the minimal amount necessary, but the excess has no deleterious action.

The present investigation has shown that reduction in surrounding $K^+$ causes progressive decrease in rate and amplitude of contraction. This is due to hyperpolarisation of the plasma membrane on account of diminution in outside $K^+$. As in $K^+$-free Ringer-Locke solution, spontaneous rhythmicity was still present, it is suggestive that the role of external $Na^+$ and $Ca^{++}$ is comparatively more than that of $K^+$ in maintaining the spontaneous rhythmicity of duodenum.

The rise in tone on lowering the concentration of $K^+$ observed in our experiments could possibly be due to the relative increase in surrounding $Ca^{++}$, because Davson (1959) has remarked that the action of a low $K^+$ may be mitigated by reducing the concentration of $Ca^{++}$; while the effect of excess of $K^+$ can also be counteracted by raising the concentration of $Ca^{++}$.

The "K-paradox" observed in duodenal muscle due to external $K^+$ is similar to the one observed by Libbrecht (1921) in cardiac muscle. This is possibly due to the duodenum, having "tried" to compensate for lack of $K^+$, finds itself overcompensated when a normal $K^+$ concentration is supplied and has to stop temporarily from an excess $K^+$ effect until re-adaptation is complete.

In 1928, Clark, Perival and Stewart found that mechanical response of frog's heart varied directly with the concentration of $Ca^{++}$; greater the $Ca^{++}$ in the surrounding medium, greater is the contraction. Recently Bozler (1960) has observed that spontaneous activity of the frog's stomach ceased after immersion in isotonic

sucrose solution, and was restored by addition of small amount of $CaCl_2$. We have also observed diminution in rate, rhythm, tone and amplitude of duodenal contraction with diminution of $Ca^{++}$. In $Ca^{++}$-free modified Ringer-Locke solution irregularities appear with the ultimate loss of spontaneous rhythmicity, indicating that the $Ca^{++}$ is essential for normal spontaneous activity of rabbit's duodenum.

The diminution in muscular activity is possibly due to the reduction of external $Ca^{++}$, disturbing the $Ca^{++}$-gradient between the outer and inner regions of the cell making the tissue less excitable and which ultimately loses spontaneous rhythmicity in absence of $Ca^{++}$ (Heilbrunn and his associates, 1956).

The present work has indicated that $Na^+$ and $Ca^{++}$ are necessary for spontaneous rhythmicity of rabbit's duodenum; while extracellular $K^+$ has a relatively minor role.

The amount of $Na^+$ in Ringer-Locke solution can be easily reduced to 0.60%. This may conserve the energy of the muscle used in pumping out the $Na^+$.

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