A Link Between the Effect of Procaine on Oxygen Consumption and Its Effect on The Mechanical Activities of The Rat Ileum Smooth Muscle

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ABSTRACT

This study has been carried out to know the effect of procaine on the spontaneous rhythmic contraction of the smooth muscle of the rat ileum. Also experiments were done in this study to clarify the effect of this drug on O₂ consumption in the whole muscle and in the mitochondria and the membrane vesicles. The aim of these experiment was a trial to understand the mechanism by which procaine exerts it's effect on this kind of smooth muscle. The drug has been used in different levels of concentration. It was found that the low and moderate concentrations enhanced the mechanical activity and increased O₂ consumption. The very high concentrations of the procaine caused reverse action. It brought about immediate relaxation and highly reduced O₂ consumption.

These results may indicate that procaine exerts its effect on this muscle through interaction with Ca²⁺ influx through the cell membrane. The correlation between the effect of the drug on the mechanical activity and on O₂ consumption may pin point to a link between these two parameters of the procaine on spontaneous contraction and the O₂ consumption in this kind of smooth muscle.

Keywords: Smooth muscle ; Procaine ; O₂ Consumption

INTRODUCTION

Procaine is a compound which is believed to specifically inhibit the Ca²⁺ induced Ca²⁺ releasing mechanism (Endo, 1977). The effect of procaine on the mechanical and electrical activities of the excitable tissue including smooth muscles has been investigated by (Washizuy, 1968b; Kurihara, 1973; Kurihara, 1975; Kurihara and Sakai; 1976 Kuriyama et al., 1998; Saad, 2008). Procaine was found to cause a rise in tone and enhance spontaneous rhythmic contractions in the urinary bladder smooth muscle of the bull frog (Kurihara1973) and the taenia coli of the guinea pig (Magaribuchi et al., 1973). This action of the drug attributed to a reduction of the membrane conductance to K⁺.

It has been suggested that the stimulation of spontaneous contractions is related to the Ca²⁺ spikes which may be induced by the interaction between procaine and Ca²⁺ at the membrane
sites (Shanes et al., 1959; Aceves and Machne, 1963; Blaustein and Goldman, 1966; Washizuy, 1968b). On the other hand Feinstein (1966) and Feinstein and Paimer (1969), reported that in the smooth muscle, procaine showed non specific inhibition of contraction, and its effect was attributed to the inhibition of \( \text{Ca}^{2+} \) permeation through the cell membrane which believed to release bound \( \text{Ca}^{2+} \) on the intracellular membrane system. In contrast to the effect of procaine on the taenia coli, Sakai and Iizuka (1972) reported that the release of bound \( \text{Ca}^{2+} \) induced by rapid cooling was inhibited by caffeine. The effect of procaine on the isolated smooth muscle preparation of the dog coronary artery was studied by Imai et al., (1984). A dose related relaxation of the K\(^+\) contracture was observed with procaine.

On the other hand another studies on different muscles found a correlation between the effect of some agents on the mechanical activities and on the oxygen consumption (Al-Badran, 1989; Eiji and Koji, 2002; Dash et al., 2005; Euzinga et al., 2006 Jurgen et al., 2007).

In the present study, the effect of procaine on the mechanical activity and on the \( \text{O}_2 \) consumption of the rat ileum by whole muscle, as well as in isolated mitochondria and membrane vesicles was investigated as a trial to find out if there is a link between the action of procaine on \( \text{O}_2 \) consumption and the mechanical activity which may help to understand the mechanism by which procaine exerts its effects on the mechanical activities of this kind of smooth muscle.

**EXPERIMENTAL**

**Materials And Methods:**

**Preparation Of Homogenates And Determination Of \( \text{O}_2 \) Consumption:**

Homogenates were prepared from the pooled ileum muscles of adult (two months old) rats using the same standard procedure described by (Gabella and Raeymaekers, 1976). To determine the weight of the mitochondria and membrane vesicles the protein was determined using the Biuret-reagent which was used by (Gabella and Raeymaekers, 1976). The phosphate in 1 mg protein also determined (which used as indicator for enzyme activity in the mitochondria and membrane vesicles before measuring the \( \text{O}_2 \) consumption). The procedure was essentially performed as used by (Black et al., 1965) with some modifications. The modification was in the incubation medium which had the following contents in mM (NH\(_4\))\(_6\) MO\(_7\)O\(_{24}\) - 4H\(_2\)O, 1; distilled H\(_2\)O, 2.4; Steanneous chloride diluted solution, 0.2; final volume of the incubation medium was 5 ml by adding distilled water and left for 10 min. UV / Visible scanning Spectrophotometer (4049) from PG Instruments limited Company UK was used for the determination of the phosphate at wave length of 660 nm. \( \text{O}_2 \) consumption was measured by manometric techniques using Warburge apparatus as described by (Umbreit, et al., 1964).
and Al-Badran, 1989). After dissecting the animal the ileum cleaned and the muscle dissected to pieces each of 0.5gm. The preparations were incubated in Krebs solution for 2 hour and continuously aerated and kept at 37°C. At the end of the incubation period, the preparation was placed in a stirred chamber with 3 ml of Krebs bicarbonate solution containing 11.5 Mm glucose (pH 7.3). The chambers were sealed and connected to manometer tubes contains Brodies fluid to zero level, then the manometer openings closed. After each 5 minutes the change in the manometric fluid level was recorded. O2 consumption calculated and expressed as micro litre O2 consumed per/hour/mg protein. The same procedures were followed for O2 consumption by the mitochondria and the membrane vesicles except the weight of the fraction used in each chamber was (1) mg protein.

The Mechanical Activity Experiments:

Rats of both sexes aged about 2 months were used in the present experiments. The ileum dissected to strips (about 2cm long). All the muscle strips were incubated for 20 minutes in Kreb's solution containing (mM). NaCl, 121; KCl, 5.9; MgCl2, 1.2; CaCl2, 2.5; NaHCO3, 15.5; Na2PO4, 1.2 and glucose, 11.5, at pH 7.3, continuously aerated with 95% O2 and 5% CO2 at 37°C. For tension recording the preparation was mounted vertically in a 50 ml organ bath which continuously aerated and kept at 37°C.

The preparation connected via a conventional strain gauge to standard Kymograph pen recorder from Griffin Company UK. Details of the methods used have been published elsewhere (Syson & Huddart, 1973 and Saad, 1980). The pH of all solutions were 7.3 as measured with a PW 9420 pH meter. Pure Procaine hydrochloride was obtained from BDH Biochemical Company UK.

RESULTS AND DISCUSSION

Effect Of Procaine On Mechanical Responses:

Before studying the effect of procaine on the mechanical activity, normal spontaneous contractions of the rat ileum smooth muscle were established in normal Krebs solution as shown in (Fig. 1 a).

When low concentrations of procaine (0.1mM, 0.5mM & 1.0 mM) were added to normal Krebs solution, spontaneous rhythmic contractions were increased in the basic tension and in the height of contractions (Fig.1 b,c,d).

The application of moderate concentrations of the drug (3mM,5mM & 8mM) caused further increase in the tension and the height of contractions and led to contractures then return to a regular rhythmic contractions (Fig. 2).
It has been observed that with these concentrations of procaine, the muscle attained maximum peak tension and gradually returned to its basic tension level but with 8 mM of procaine the decline in tension and irregularity of the spontaneous contraction was faster than in the (3mM and 5mM) concentrations of the drug.

At higher concentration of procaine (10mM), the tone is increased and promptly caused contracture but this was not maintained, and eventually the contracture and the tension was decline gradually and the rhythmic contractions finally disappeared and reached to complete relaxation as shown (Fig.3a). when a very high concentration of the drug (15mM) applied to the preparation immediate relaxation was observed within few seconds as can be seen in (Fig.3b).

**Effect Of Procaine On Oxygen Consumption**

The O2 consumption in the whole muscle and in the fractions (mitochondria and the membrane vesicles) were established in normal physiological conditions prior studying the effect of procaine. This drug showed a paradox effect on O2 consumption depending on its concentration. O2 consumption was elevated in the whole muscle when low and medium concentrations (0.1mM & 3mM) of procaine were introduced to the incubation medium (Fig. 4). The same concentrations caused an increase in the mechanical activities. In contrast, the higher concentration of procaine (15mM), caused a very clear reduction to about (65%) in the O2 consumption. The same concentration led to abolishing the mechanical activity.

The effect of the drug on O2 consumption on the sub-cellular fractions was tested. As it can be seen in (fig. 5 and 6) the drug has different effects depending on the concentration used. It was found that the low (0.1mM) and moderate concentrations (3Mm) enhanced the O2 consumption where as the high concentration had the opposite effect in both the mitochondria and in the membrane vesicles.

The increase in O2 consumption in the membrane vesicle by low and medium concentrations of the drug was slightly lower than that in the mitochondria.

It has been suggested that in smooth muscle Ca2+ ions and procaine compete in their action on the membranous conductance mechanism. More over, Ca++ have an important role in spike generation (Blaustein & Goldman, 1966; Brading et al., 1969; Bulbring and Tonita, 1970).

In the present study it was found that low and moderate concentrations of procaine induced an increase in the tension and the height of the spontaneous contraction. This effect of procaine may support the above finding. Furthermore, procaine (3&8mM) caused contracture in this muscle. This result may further support the hypothesis that enhancement of contractions by procaine may be due to increase of Ca2+ ion entry through the cell membrane of the rat ileal smooth muscle (Huddart et al., 1984).
It was also reported that procaine cause a rise in tone of the Taenia coli of the guinea pig, and induced a marked contracture in bull frog urinary bladder smooth muscle (Kurihara, 1973). This was considered to be dependant on Ca\(^{2+}\) influx through the cell membrane and Ca\(^{2+}\) mobilization from sequestered sites (Urakawa and Holland, 1964; Imai and Taked, 1967; Antio et al., 1970). The hypothesis, that the external Ca\(^{2+}\) entry induces Ca\(^{2+}\) release from the intracellular storage sites supported by (Endo, 1977; Kunihiko et al., 1998).

In dog coronary artery procaine induced relaxation of the K\(^{+}\) contracture (Imai et al., 1984; Spedding and Berg, 1985). Thus on the bases of these results, it may be reasonable to assume that the relaxation induced by very high concentrations of procaine in the rat ileum was due to an inhibition of Ca\(^{2+}\) induced Ca\(^{2+}\) release from the intracellular storage site. These considerations mentioned above, were further confirmed by the experiments on the \(O_2\) consumption. It has been found in this study that the effect of the procaine on the \(O_2\) consumption depends on the concentration of the drug used in the experiments in the whole muscle as well as in the mitochondria and the membrane vesicles. It was found that same concentrations of procaine that caused stimulation of the mechanical activities also inhibiting effect. The results of these experiments show a clear correlation between the action of the procaine on the mechanical activity and its effect on \(O_2\) consumption. The effect of Procaine on the rat ileal smooth muscle is similar to the effect of quinine on the smooth muscle of the golden hamster and mice (Al-Badran, 1989).

**CONCLUSION**

These finding may indicate to a clear link between \(O_2\) consumption and the mechanical activity. The mechanism possibly brought about by procaine effect on the availability of certain level of free Ca\(^{2+}\) inside the cells that required for contraction.
Fig. (1): Normal spontaneous rhythmic contraction in normal Krebs solution (a) and (b, c, d) the effect of low concentrations (0.1, 0.5 and 1mM) respectively of the procaine on the mechanical response of the rat ileum.
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Fig (2): Effect of high conc. of procaine on the mechanical response of the rat ileum (a, b, c) show the application of 3, 5 and 8 mM respectively to normal Krebs.

Fig (3): Effect of very high concentration of procaine on the mechanical response changes induced by 10 and 15 mM procaine (a, b) respectively.
Fig. (4) The effect of procaine on O₂ consumption in the whole smooth muscle of rat ileum

Fig. (5) The effect of procaine on O₂ consumption in the mitochondria.
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Fig. (6) The effect of procaine on O$_2$ consumption in membrane vesicles

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<tr>
<th>Concentration (mM)</th>
<th>μL O$_2$/mg protein dry weight/hour</th>
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<tbody>
<tr>
<td>Normal</td>
<td>7.19</td>
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<tr>
<td>0.1 mM</td>
<td>8.36</td>
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<tr>
<td>3 mM</td>
<td>9.26</td>
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<td>15 mM</td>
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REFERENCES


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