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The effects of isoprenaline and phenylephrine on oxygen consumption in isolated smooth muscle

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Summary

1. Simultaneous measurements of oxygen consumption and mechanical activity have been made in isolated preparations of guinea-pig taenia coli, rabbit abdominal aorta and the longitudinal muscle of rabbit duodenum.

2. Mean resting oxygen consumption was greater in mechanically-active preparations (taenia coli and duodenum) than in the aorta which showed no spontaneous activity.

3. The relaxation produced by isoprenaline in guinea-pig taenia coli and rabbit duodenum was accompanied by a dose-dependent fall in oxygen consumption.

4. The relaxation produced by phenylephrine in guinea-pig taenia coli was accompanied by a dose-dependent fall in oxygen consumption whereas, in the rabbit duodenum, the transient fall in tension was not accompanied by any significant change in oxygen consumption.

5. The increase in tension produced by phenylephrine in rabbit abdominal aorta was accompanied by an increase in oxygen consumption.

6. Changes in mechanical activity and oxygen consumption produced by isoprenaline and phenylephrine were antagonized by propranolol and phentolamine respectively.

7. It is concluded that the variations in oxygen consumption associated with spontaneous or drug-induced mechanical changes are simple reflections of altered mechanical activity. The possibility of additional direct metabolic actions of isoprenaline and phenylephrine is discussed.

Introduction

Changes in the amounts of adenosine triphosphate (ATP), creatine phosphate and glucose-6-phosphate which accompany the mechanical effects of isoprenaline and phenylephrine in smooth and cardiac muscle have been described by Weston (1971a). When mechanical effects were mediated by β-adrenoceptors, significant changes in the amounts of ATP and creatine phosphate were observed. An increase in mechanical activity was accompanied by a reduction in energy-rich phosphate compounds whilst decreased mechanical activity was associated with an increase in the amounts of ATP and creatine phosphate. In contrast, similar mechanical effects mediated by α-adrenoceptors were unaccompanied by detectable changes in the amounts of ATP, creatine phosphate and glucose-6-phosphate.

In both intestinal and vascular tissues, aerobic mechanisms play an important

part in the synthesis of ATP (Bueding, Bülbring, Gercken, Hawkins & Kuriyama, 1967; Somlyo & Somlyo, 1968). For this reason, measurement of changes in tissue oxygen consumption should give some indication of fluctuations in oxidative ATP synthesis. This paper describes the results of experiments in which the changes in oxygen consumption which accompanied the mechanical effects of isoprenaline and phenylephrine were measured in intestinal and vascular tissues. It was hoped that such experiments would assist in the interpretation of the biochemical changes accompanying mechanical responses mediated by α- and β-adrenoceptors.

Methods

Preparation of isolated tissues

Experiments were performed with guinea-pig taenia coli, rabbit aorta and the longitudinal muscle strip of rabbit duodenum. These tissues were prepared as described by Weston (1971a). The technique of Bülbring & Kuriyama (1963) was used to provide standard conditions within a group of tissues by stretching each tissue to a given length in relation to its weight (W:L ratio, mg/mm).

Experimental procedures

The concentrations of the agonists isoprenaline and phenylephrine and the antagonists propranolol and phentolamine used in the experiments were the same as those used in a previous study (Weston, 1971a) and were derived from conventional tissue bath experiments.

The approximate ED20 and ED80 of each agonist on each tissue was first determined. This involved measurement of the reduction in amplitude of spontaneous mechanical activity in rabbit duodenum and of the increase in tension in rabbit aorta. In taenia coli, the ability to reduce the size of an acetylcholine spasm (100 nM$\frown$ED90) was used. Experiments with increasing concentrations of phentolamine or propranolol allowed the calculation of the pA_{100} of antagonist against agonist and also enabled the concentration of antagonist which reduced the response to the ED80 of agonist to that to the ED20 to be calculated. The negative logarithm of this antagonist concentration was designated the pA_R. These values (ED20, ED80, pA_{100} pA_R) were then used in the oxygen consumption experiments. All experiments with phenylephrine were conducted in the presence of a pA_{100} of propranolol against isoprenaline. All experiments with isoprenaline were conducted in the presence of a pA_{100} of phentolamine against phenylephrine.

Measurement of oxygen consumption

Oxygen consumption was measured by a modified stop-flow technique (Bülbring, 1953). Tissues were mounted in a thermostatically-controlled perspex chamber, constructional details of which have been described (Weston, 1970). One end of the tissue was fixed within the chamber whilst the other was attached to a cotton thread which passed out of the apparatus and was connected to a force-displacement transducer (Ether). The Krebs solution in the chamber could be agitated with a small magnetic stirrer and gassed with either 5% CO_2 in O_2, or 5% CO_2 in N_2.

The oxygen content of the Krebs solution was measured by an oxygen electrode

(Beckman) inserted into the chamber and connected to a gas analyser (Beckman). The output of the analyser, together with the output of the force-displacement transducer were simultaneously displayed on two channels of a potentiometric recorder (Rikadenki).

A length of tissue (2–3 cm) was placed in the apparatus and allowed to equilibrate in Krebs solution bubbled with 5% CO_2 in O_2 for 1 h, after which it was stretched to an appropriate W:L ratio. Bubbling was stopped after 10 min and after a further 2 min measurement of oxygen consumption was begun, the Krebs solution being agitated with the magnetic stirrer. The 2 min delay was allowed because it was observed that, immediately after bubbling had ceased, there was a marked fall in the output of the electrode. This was interpreted as evidence of supersaturation of the Krebs solution with oxygen resulting from bubbling with a gas under pressure.

Measurements of oxygen consumption were usually made for a period of 5 minutes. The tissue was then exposed to acetylcholine, isoprenaline or phenylephrine by injecting a suitable quantity of drug contained in Krebs solution (0·05 ml) into the chamber. After the effect of the drug had been measured, bubbling with 5% CO_2 in O_2 was resumed.

Electrode calibration

The electrode was calibrated in each experiment by measuring its output when immersed in Krebs solution at 37° C and equilibrated with 5% CO_2 in O_2, with 5% CO_2 in N_2 and with air. The activity coefficient of oxygen in Krebs solution was determined (Dixon & Kleppe, 1965) allowing the oxygen concentration of the solution to be calculated. The response of the electrode was found to be linear between the upper and lower limits of oxygen tension used.

Calculation of oxygen consumption

The amount of oxygen in the Krebs solution in the chamber was reduced by: (a) loss to the atmosphere via holes in the screw cap of the tissue bath, (b) utilization by the tissue. The former was determined over a 5 min period before the tissue was placed in the chamber. Tissue oxygen consumption was determined by subtracting the amount of oxygen lost from the bath in a given time in the absence of the tissue from the amount lost in the presence of the tissue in the same time.

Wherever possible, drug-induced changes were measured over a period of 1 minute. In the case of very rapid responses, however, this was not possible and, in these instances, the values of oxygen consumption quoted were obtained by extrapolation of the responses to 1 minute.

Injection artefacts

In some experiments, the injection of Krebs solution containing the drug under study produced an artefact in the record of oxygen consumption. This consisted of a rapid change in the output of the electrode and was probably caused by a difference in temperature or oxygen tension between the drug solution and bath contents.

Drugs and solutions

The Krebs solution used had the following composition (mM): Na^+ 143·0, K^+ 5·9, Ca^{++} 2·5, Mg^{++} 1·2, Cl^- 125·0, HCO_3^- 25·0, SO_4^{--} 1·2, $H_2PO_4^-$ 1·2, dextrose 11·1. The pH of this solution was 7·4 while bubbling with 5% CO_2 in O_2.

Drugs used were acetylcholine chloride (B.D.H.); (−)-isoprenaline bitartrate dihydrate (Ward Blenkinsop); phentolamine hydrochloride (Ciba); (−)-phenylephrine hydrochloride (Boots); (±)-propranolol hydrochloride (I.C.I.).

For injection into the tissue chamber, drug dilutions were made in Krebs solution pre-warmed to 37° C and bubbled with 5% CO_2 in O_2.

Statistical methods

Student's *t* test (two-tailed) applied according to Goldstein (1967) was used to measure the probability of differences between mean responses arising by chance. The measure of variability used is the standard error.

Results

Spontaneous activity

All segments of taenia exhibited characteristic spontaneous mechanical activity consisting of waves of tension whose frequency, duration and amplitude varied with the degree of stretch. In preparations stretched to W:L=0·5 (equivalent to a mean resting tension of 3·8 g), there was an increase in oxygen consumption associated with the rising phase of each tension wave (Fig. 1). Oxygen consumption

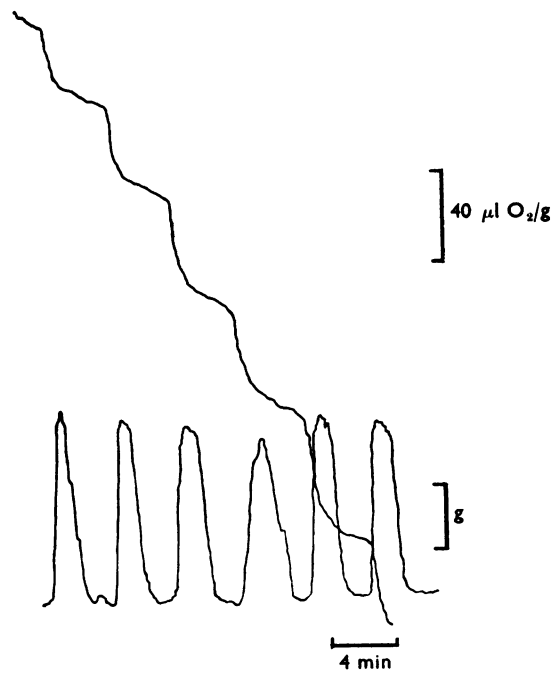

FIG. 1. Guinea-pig taenia coli. Oxygen consumption and mechanical activity in a segment stretched to W:L=0·5 in Krebs containing phentolamine (1·5 µM). Upper trace: oxygen consumption (µl oxygen/g wet weight of tissue). Lower trace: spontaneous mechanical activity.

was low during the periods of mechanical quiescence between tension waves. Measurements were made in Krebs solution containing (a) the pA_{100} of phentolamine (1·5 μM) and (b) the pA_{100} of propranolol (2·4 μM). The mean oxygen consumption derived from both groups of experiments measured from the start of one tension wave to the start of the next was $12·8 \pm 0·9$ μl/g wet weight of tissue/min (30 experiments). After stretching to W:L=0·5, resting tension was not maintained and usually declined to zero within 1 hour.

In preparations stretched to W:L=0·3 (equivalent to a mean resting tension of 6·1 g), the frequency of tension waves was reduced but their duration was sufficiently prolonged to give a stable baseline against which the action of relaxants could be investigated. At W:L=0·3, the mean oxygen consumption was ($15·5 \pm 1·1$ μl/g wet weight of tissue)/min (30 experiments). The taenia coli is depolarized by a W:L ratio of 0·4 (Bülbring & Kuriyama, 1963) and this is likely to have occurred in the present study.

The longitudinal muscle strips of rabbit duodenum exhibited waves of tension similar to those generated by intact, isolated duodenum. In preparations stretched to W:L=1·5 (equivalent to a mean resting tension of 1·4 g), oxygen consumption was ($14·0 \pm 1·8$ μl/g wet weight of tissue)/min (30 experiments). This value was derived from experiments conducted in the presence of (a) the pA_{100} of phentolamine (2·5 μM) and (b) the pA_{100} of propranolol (4·75 μM). Fluctuations in oxygen

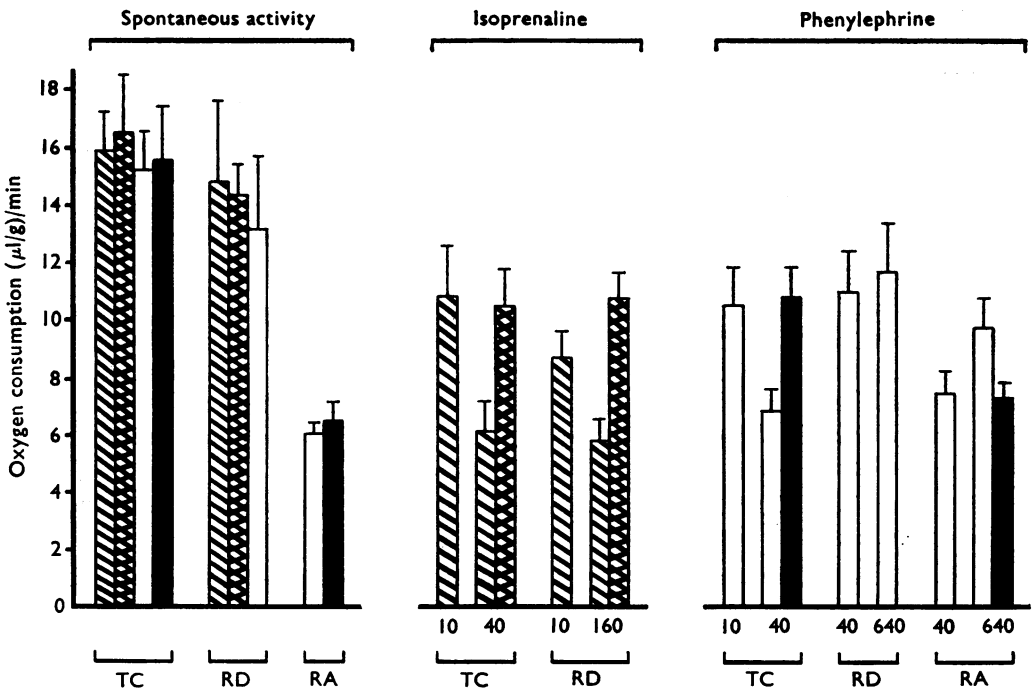

FIG. 2. Effects of isoprenaline and phenylephrine on oxygen consumption in guinea-pig taenia coli (TC) at W:L=0·3; longitudinal muscle of rabbit duodenum (RD) at W:L=1·5; and rabbit abdominal aorta (RA) at W:L=0·8. Each vertical bar represents the mean of at least fifteen experiments ± S.E. conducted in Krebs solution containing phentolamine pA_{100} ▩; phentolamine pA_{100} + propranolol pA_R ▩; propranolol pA_{100} □; propranolol pA_{100} + phentolamine pA_R ■. See text for antagonist concentrations. Numbers on abscissae refer to concentrations (nM) of isoprenaline or phenylephrine to which tissues were exposed.

consumption associated with the rising and falling phases of each mechanical wave were not detected.

In preparations of rabbit abdominal aorta stretched to W:L=0·8 (equivalent to a mean resting tension of 1·5 g), oxygen consumption was (6·0 ± 0·5 μl/g wet weight of tissue)/min (18 experiments). All experiments with this tissue were conducted in the presence of an estimated pA_{100} of propranolol (2 μM).

The results of oxygen consumption measurements made during spontaneous mechanical activity in all three tissues are summarized in Fig. 2.

Effects of isoprenaline

When a segment of taenia coli (stretched to W:L=0·5) was exposed to isoprenaline (10 nM⌒ED20 and 40 nM⌒ED80) during the rising phase of a tension wave, the wave was inhibited and oxygen consumption was reduced to an amount not significantly different from that occurring during the periods of quiescence between tension waves (15 experiments, $P \geqslant 0{\cdot}3$). Administration of isoprenaline during a quiescent period when resting tension was zero produced no significant change in oxygen consumption (15 experiments, $P \geqslant 0{\cdot}4$).

Because of the irregular mechanical activity of the taenia, acetylcholine (100 nM⌒ED90) was used to obtain a standard increase in tension against which isoprenaline-induced relaxations and associated changes in oxygen consumption could be measured. In this concentration, acetylcholine caused an increase in oxygen consumption from 11·7 ± 0·8 to 17·3 ± 0·7 (μl/g wet weight of tissue)/min (15 experiments). However, the injection of isoprenaline and acetylcholine consecutively into the tissue chamber was difficult to achieve without the production of artefacts in the oxygen consumption record. For this reason, further experiments with isoprenaline were performed with preparations stretched to W:L=0·3. Under these conditions, isoprenaline produced a concentration-dependent reduction in tension and in oxygen consumption, effects which were reduced in the presence of propranolol (100 nM $\equiv pA_R$).

When longitudinal muscle strips of rabbit duodenum were exposed to isoprenaline (10 nM⌒ED20 and 160 nM⌒ED80), a concentration-dependent reduction in mechanical activity and in oxygen consumption occurred. The changes in mechanical activity were prolonged, there being little tendency for activity to return to control levels in the presence of isoprenaline. The effects of isoprenaline were reduced in the presence of propranolol (650 nM $\equiv pA_R$).

In the rabbit abdominal aorta, isoprenaline (up to 1 μM) produced no change in tension or in oxygen consumption.

The effects of isoprenaline on oxygen consumption in guinea-pig taenia coli and in the rabbit duodenum are shown in Fig. 2.

Effects of phenylephrine

In the taenia coli stretched to W:L=0·5, the effects of phenylephrine (10 nM ⌒ED20 and 40 nM⌒ED80) were very similar to those produced by isoprenaline. When phenylephrine was administered during the rising phase of a tension wave, the wave was inhibited and oxygen consumption was reduced to an amount not significantly different from that occurring during the quiescent periods between tension waves (15 experiments, $P \geqslant 0{\cdot}4$). However, exposure to phenylephrine

during a quiescent period produced no significant change in tension or in oxygen consumption (15 experiments, $P \geqslant 0{\cdot}3$). In preparations stretched to W:L=0·3, phenylephrine produced a concentration-dependent reduction in tension and in oxygen consumption, effects which were antagonized by phentolamine (70 nM$\equiv$ pA_R).

When longitudinal muscle strips of rabbit duodenum were exposed to phenylephrine (40 nM$\frown$ED20 and 640 nM$\frown$ED80), a concentration-dependent reduction in tension was produced without a significant change in oxygen consumption. The reduction in tension was of a transient nature, mechanical activity returning to control levels in the presence of phenylephrine.

In rabbit abdominal aorta, exposure to phenylephrine (40 nM$\frown$ED20 and 640 nM$\frown$ED80) produced a concentration-dependent increase in tension and in oxygen consumption, changes that were antagonized by phentolamine (140 nM$\equiv pA_R$).

The effects of phenylephrine on oxygen consumption are summarized in Fig. 2.

Discussion

On the basis of their experimental observations, Bueding & Bülbring (1964) suggested that the inhibitory action of adrenaline in intestinal smooth muscle was produced by an increase in the rate of cellular metabolism. Later, adrenaline was shown to increase the amounts of ATP and creatine phosphate in guinea-pig taenia coli, an action which required oxygen but which was not dependent on carbohydrate stores within the cell (Bueding *et al.*, 1967). However, a parallel investigation showed that relaxation of guinea-pig taenia coli by adrenaline was accompanied by a decreased tissue oxygen consumption (Bülbring & Golenhofen, 1967). This suggested that the observed increase in energy-rich phosphate compounds was a secondary effect resulting from diminished mechanical activity and a decrease in the utilization of ATP and creatine phosphate.

The results of the present investigation, together with those of previous workers, suggest that drug-induced changes in oxygen consumption in smooth and cardiac muscle are the result of altered mechanical activity and not of direct metabolic action. An increase in oxygen consumption accompanied the increase in tension produced spontaneously (Bülbring, 1953; Bülbring & Golenhofen, 1967; this study), by acetylcholine (Bülbring, 1953; this study), by histamine (Bülbring, 1953), by potassium ions (Saito, Sakai, Ikeda & Urakawa, 1968), and by activation of α-adrenoceptors (this study) and β-adrenoceptors (Williamson, 1966). A decrease in tension produced spontaneously (Bülbring & Golenhofen, 1967; this study) or by activation of α- or β-adrenoceptors was accompanied by a reduction in oxygen consumption.

Catecholamines are known to stimulate metabolism in several tissues (Exton & Park, 1966; Steinberg, 1966), and the above observations do not eliminate the possibility that some direct metabolic component was masked by the larger changes due to muscular activity. However, isoprenaline and phenylephrine (this study) and adrenaline (Bülbring & Golenhofen, 1967) failed to produce changes in oxygen consumption when administered during periods of mechanical quiescence in guinea-pig taenia coli. These observations further suggest that, in mechanically active preparations, the metabolic changes were the result of altered mechanical activity and did not involve a direct metabolic action of the drugs.

In rabbit duodenum, no change in oxygen consumption was associated with phenylephrine-induced inhibition of mechanical activity. It is possible that the inhibitory response in this tissue was too transient to be reflected in the oxygen consumption record. Bowman & Hall (1970) have suggested that the action of phenylephrine might consist of an inhibitory followed by an excitatory action and it is possible that such an effect could explain the failure to observe a change in oxygen consumption.

With the exception of α-adrenoceptor activation in rabbit duodenum, the changes in oxygen consumption observed in guinea-pig taenia coli, rabbit duodenum and rabbit aorta could be predicted from the excitatory or inhibitory nature of the mechanical event. In contrast, there was a marked difference between the changes in energy-rich phosphate compounds associated with activation of α- and β-adrenoceptors in these tissues (Weston, 1971a). Activation of β-adrenoceptors produced an increase in ATP and creatine phosphate associated with relaxation and a decrease associated with excitation. Together with the changes observed in oxygen consumption, this suggests that the metabolic changes reflect the requirements of the muscle for energy-rich phosphate compounds generated by oxidative synthesis. Mechanical events mediated by α-adrenoceptors were unaccompanied by detectable changes in the amounts of ATP and creatine phosphate in spite of the fact that tissue oxygen consumption followed a pattern similar to that associated with β-adrenoceptor activation. These differences are not easy to explain although the work of Jenkinson & Morton (1965, 1967) on potassium ion fluxes and of Bülbring & Tomita (1969) on membrane conductance changes in intestinal smooth muscle has also shown differences in the mechanisms underlying α- and β-adrenoceptor-mediated responses.

Recent work has shown that recovery from the inhibitory effects of phenylephrine in rabbit duodenum is accompanied by an increase in spike frequency in contrast to recovery from the effects of isoprenaline (Weston, 1971b). This supports the suggestion by Bowman & Hall (1970) that α-adrenoceptor-mediated responses may consist of inhibitory and excitatory components and indicates the need for more detailed studies on the biochemistry of smooth muscle to resolve these problems.

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