Br. J. Pharmac. (1968), **34**, 493–498.

Effects of metabolic inhibitors on contraction of rabbit detrusor muscle

D. M. PATON

Department of Pharmacology, University of Alberta, Edmonton, Alberta, Canada

1. The contractile responses of rabbit detrusor muscle strips to acetylcholine are reversibly depressed but not abolished by 2,4-dinitrophenol (0.1–1.0 mM) provided that D-glucose or D-mannose is present. L-glucose, D-2-deoxyglucose, D-galactose, D-fructose, D-xylose, maltose, lactose and sucrose are ineffective in this respect.

2. The contractile responses are greatly reduced by iodoacetic acid (0.1–1.0 mM) in 30–60 min and eventually abolished. The depressant effect of iodoacetic acid (0.25 mM) can be partially reversed or retarded by pyruvate (20 mM).

3. It is concluded that the energy for contraction of the detrusor muscle can be supplied by glycolysis alone. Because both D-glucose and pyruvate can be utilized, it is assumed that the glycolytic route and Krebs cycle are available in the detrusor muscle cells.

Evidence that smooth muscle cells from different sites require metabolic energy for contraction and the maintenance of normal excitability has been obtained in several studies. Metabolic inhibitors alter electrical and mechanical activity of intestinal and uterine smooth muscle (Axelsson & Bülbring, 1961 ; Marshall & Miller, 1964). The mechanical responses of vascular smooth muscle can be maintained during anoxia by glycolysis ; however, in the presence of oxygen they appear to be maintained by a non-glucose source (Shibata & Briggs, 1967). Acetylcholine-induced contractions of immature rat uterine horns are rapidly abolished by iodoacetic acid but persist for prolonged periods in the presence of dinitrophenol, provided that D-glucose is present (Paton, 1968a).

Information on the role of metabolic factors in the contraction of the urinary bladder is limited, although recently the utilization of glucose by normal and denervated bladder muscle has been described (Rohner, Komins & Schoenberg, 1967). In the investigation reported here, dinitrophenol and iodoacetic acid were utilized to study the role of metabolic factors in the contraction of the detrusor muscle. Rabbit isolated detrusor muscle strips were chosen as the test organ as information is available on certain aspects of their physiology and pharmacology (Ursillo & Clark, 1956 ; Ursillo, 1961 ; Paton, 1968b, c). A preliminary account of part of this work has been presented elsewhere (Paton, 1968d).

Methods

Technique for recording contractions of detrusor muscle

Male New Zealand rabbits (1.5–3.5 kg) were killed by a blow on the head; the urinary bladders were excised rapidly, rinsed in Krebs-Ringer solution at room temperature and dissected free of surrounding connective tissue. Longitudinal strips of detrusor muscle, about 2 mm wide and 15 mm long, were prepared and mounted vertically in individual baths of 10 ml. volume in Krebs-Ringer solution gassed with 95% oxygen and 5% carbon dioxide at a temperature of 37° C. Three or four strips from the same animal were used simultaneously in each experiment. Isometric contractions were recorded by Grass force displacement transducers (FT 03) connected to a Grass polygraph (Model 5D); the initial tension was 400–600 mg.

Contractions were induced by a supramaximal concentration of acetylcholine which was washed out when a maximal contraction had been attained or 3 min after administration, whichever occurred sooner. Intervals of at least 5 min were allowed between administrations of acetylcholine.

Krebs-Ringer solution of the following composition was used (mM): NaCl 115.5, KCl 4.6, $CaCl_2$ 1.5, $MgSO_4$ 1.2, $NaHCO_3$ 21.9, NaH_2PO_4 1.2, glucose 49.2.

Drugs and chemicals

The following drugs and chemicals were used: 2,4-dinitrophenol (DNP), D-glucose, sucrose, D-fructose, maltose and lactose (Fisher Scientific Company); iodoacetic acid (IAA) (Eastman Organic Chemicals); D-galactose, D-2-deoxyglucose and pyruvic acid (Sigma Chemical Company); acetylcholine chloride (British Drug Houses); and L-glucose and D-xylose (Calbiochem.). All sugars were used in a concentration of 49.2 mM. Concentrations are in mM, except that of acetylcholine, which is given as the weight of the salt.

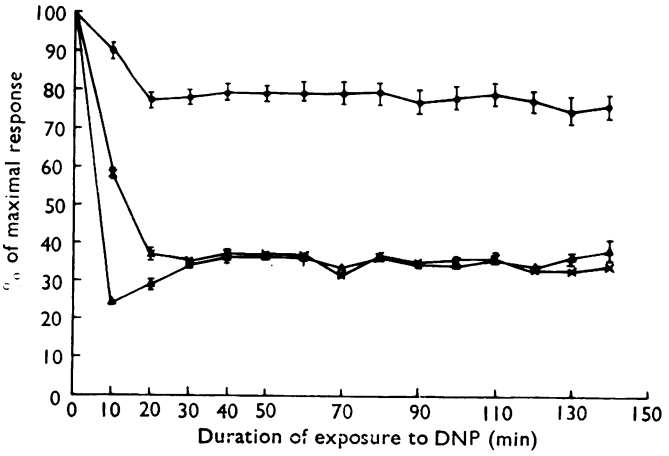

FIG. 1. Effect of dinitrophenol on the responses of rabbit detrusor muscle strips to acetylcholine. In each experiment three strips, obtained from the same animal, were used simultaneously. After isometric contractions to supramaximal concentrations of acetylcholine (10–50 μg/ml.) had been obtained (=100%) in the absence of DNP, the strips were exposed to different concentrations of DNP, 0.1 mM (●), 0.25 mM (×), and 1.0 mM (▲). The D-glucose content of the Krebs-Ringer solution was 49.2 mM, whether or not DNP was present. Each point is the mean of six observations, the vertical bars indicating ±S.E. of the mean.

Results

Effect of dinitrophenol on acetylcholine-induced contractions

In each experiment three strips from the same urinary bladder were used. After control responses to supramaximal concentrations of acetylcholine (10–50 μg/ml.) had been obtained, DNP was added to the bath to give concentrations of 0.1, 0.25 or 1.0 mM. It can be seen (Fig. 1) that DNP depressed the contractile response, the maximum effect occurring in 10–20 min ; with a concentration of 1.0 mM there was some recovery after exposure for 10 min. With all concentrations the final level of depression was reached after 30–40 min and remained constant for up to 140 min. There was no difference between the effects produced by 0.25 and 1.0 mM DNP. When DNP (1 mM) was applied for the first time, the strips responded with a small contraction of short duration (Fig. 2). On removal of DNP from the bathing medium, its depressant effects were rapidly reversed.

When strips were exposed to DNP (1 mM), in the absence of D-glucose, the response to acetylcholine was abolished ; however, when D-glucose (49.2 mM) was then added to the bathing medium, the responses recovered.

These results suggested that, in the presence of DNP, the energy requirements for contraction were supplied by glycolysis. To test this possibility the following experimental design was adopted. The strips were suspended in Krebs-Ringer solution

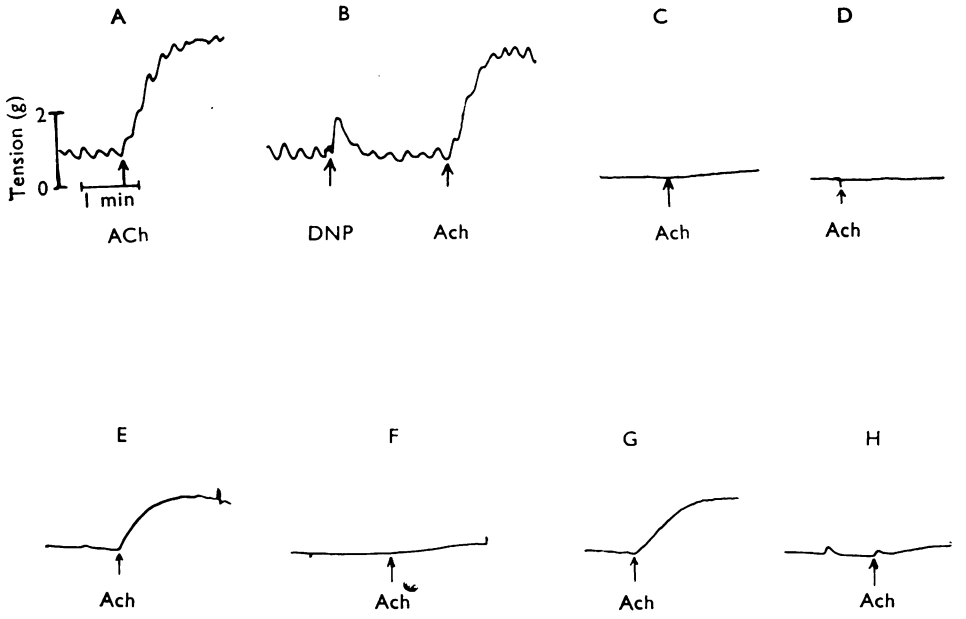

FIG. 2. Effects of various saccharides on the action of dinitrophenol on rabbit detrusor muscle strips. Acetylcholine (10 μg/ml.) was administered as indicated by the arrows. Each recording indicates a successive phase of the experiment. DNP (1 mM) was present continuously from B onwards. The saccharides were added to produce a concentration of 49.2 mM. A, Control response ; B, at first arrow, exposed to DNP in the presence of D-glucose ; acetylcholine added 2 min later (second arrow) ; C, response after 40 min exposure to DNP and sucrose ; D, response after 15 min exposure to DNP and D-fructose ; E, response after 12 min exposure to DNP and D-glucose ; F, response after 40 min exposure to DNP and D-fructose ; G, response after 10 min exposure to DNP and D-glucose ; H, response after 10 min exposure to DNP and D-2-deoxyglucose.

containing D-glucose (49.2 mM) and DNP 1 mM) and shown to contract after addition of acetylcholine. D-glucose was then replaced by another saccharide, in a concentration of 49.2 mM, and the contractile response to acetylcholine tested repeatedly. If the saccharide was unable to substitute for D-glucose it was replaced by another saccharide and the ability of this saccharide to restore the contractile response was then tested repeatedly. Finally, the responses of all strips were examined in the presence of DNP and D-glucose to ensure that the strips were still capable of contracting. It was found that only D-mannose could replace D-glucose; L-glucose, D-2-deoxyglucose, D-galactose, D-fructose, D-xylose, maltose, lactose and

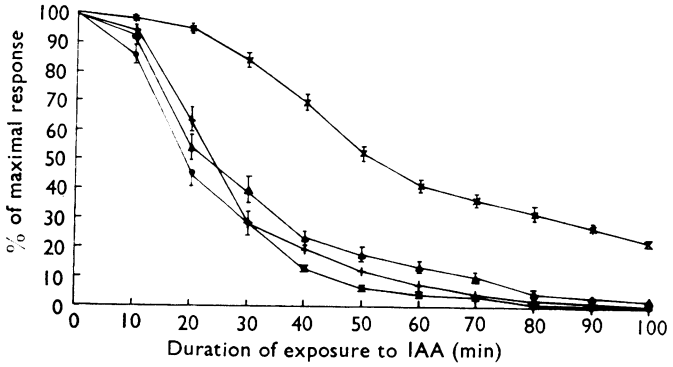

FIG. 3. Effect of iodoacetic acid on the contractile responses of rabbit detrusor muscle strips to acetylcholine. In each experiment four strips, obtained from the same animal, were used simultaneously. After isometric contractions to supramaximal concentrations of acetylcholine (10–50 μg/ml.) had been obtained (=100%) in the absence of IAA, the strips were exposed to different concentrations of IAA, 0.1 mM (×), 0.25 mM (▲), 0.50 mM (+), and 1.0 mM (●). Each point is the mean of six observations, the vertical bars indicating ±S.E. of the mean.

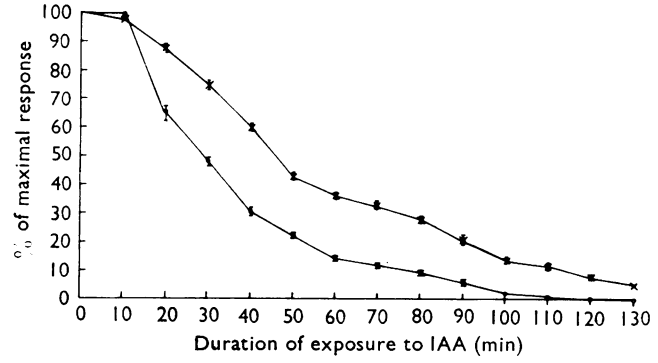

FIG. 4. Effect of iodoacetic acid and pyruvate on responses of rabbit detrusor muscle strips to acetylcholine. In each experiment two strips obtained from the same animal were used simultaneously. Isometric contractions to supramaximal concentrations of acetylcholine (10–50 μg/ml.) were obtained (=100%) in the absence of IAA and pyruvate. One strip (●) was then exposed to IAA (0.25 mM) and the other (×) to IAA (0.25 mM) and pyruvate (20 mM). Each point is the mean of six observations, the vertical bars indicating ±S.E. of the mean.

sucrose were ineffective. An example of one such experiment is shown in Fig. 2. Addition of D-glucose to the bathing medium containing DNP always rapidly restored the contractile response except after exposure to D-2-deoxyglucose when recovery was delayed.

Effects of iodoacetic acid on acetylcholine-induced contractions

Four strips from the same urinary bladder were used in each experiment. The responses to acetylcholine were tested in the absence and presence of IAA in concentrations of 0.1, 0.25, 0.5 or 1.0 mM. None of the strips contracted when first exposed to IAA. It can be seen (Fig. 3) that IAA, in all concentrations employed, produced a marked progressive depression of the contractile responses ; this depression was concentration dependent, 0.25 mM having a near-maximal effect. The effects of IAA were not reversed by washing it out. Strips thus rendered unresponsive to acetylcholine did not develop rigor but were flaccid.

To examine the effects of pyruvate on the IAA-induced depression of the contractile responses two experimental designs were followed. In the first, the depression of the responses to acetylcholine was observed in the presence of IAA (0.25) with or without pyruvate (20 mM) ; the addition of pyruvate resulted in a slower decline in the contractile response (Fig. 4). In the second type of experiment, strips were exposed to IAA (0.25 mM) and a marked depression of the contractile response was allowed to develop ; at this stage, pyruvate (20 mM) was added to the bathing medium. This resulted in an immediate but transient improvement in the response to acetylcholine.

Addition of both DNP (1 mM) and IAA (1 mM) resulted in an immediate loss of all contractile responses without the appearance of rigor ; this effect was not reversed by washing out the inhibitors.

Discussion

Rabbit isolated detrusor muscle strips were used in this study for several reasons. It was possible to separate the detrusor muscle from the trigone muscle and to obtain multiple strips from each bladder, thus providing adequate controls. Compounds could be used in concentrations which were too toxic for administration to the whole animal. Finally the pharmacology, the cation requirements for contraction and the electrophysiological properties of the preparation have already been described (Ursillo & Clark, 1956 ; Ursillo, 1961 ; Paton, 1968b, c). A disadvantage of the preparation is that diffusion is likely to be slow, as the strip consists of mucosa, muscularis and serosa.

The two metabolic inhibitors used were chosen because DNP uncouples oxidative phosphorylation (Slater, 1963) and IAA inhibits glycolysis by inhibiting the enzyme, 3-phosphoglyceraldehyde dehydroglucose (Webb, 1966).

Evidence was obtained that the energy requirements for the acetylcholine-induced contraction of the detrusor muscle can be provided, at least partially, by glycolysis. The contractile response was not abolished by DNP, provided that D-glucose was present, and D-glucose could not be replaced by a non-metabolizable sugar (sucrose, L-glucose, D-galactose, etc.). Further, in the continued presence of IAA, acetylcholine did not cause a contraction. While IAA may inhibit metabolic pathways other than glycolysis (Webb, 1966), the fact that a concentration as low as 0.25 mM had a depressant effect which was reversed, although incompletely, by pyruvate

suggests that the observed effect was at least partially an inhibition of glycolysis. Moreover, IAA probably reduced energy stores because uterine horns from oestrogen-treated rats lost 90% of their ATP stores after exposure to IAA (1 mM) (Daniel, Carroll, Robinson & Graham, 1967).

The ability of pyruvate partially to overcome the depressant effect of IAA on the contractile response suggests that the metabolic pathway of the Krebs cycle is present in the detrusor muscle cells. The fact that in the presence of DNP, sugars other than D-mannose cannot replace D-glucose may be due to an inability of the particular sugar to penetrate the membrane or to an absence of the specifie enzyme required to utilize the substrate, or both.

Investigations of other smooth muscle have shown a similar marked dependence on glycolysis. For example, exposure of rat uterine horns to IAA results in a gain of Na^+ and a loss of K^+, a rapid depression of contractile responses and an impaired retention of exogenous catecholamines; by contrast, DNP does not have these effects unless D-glucose is excluded from the bathing medium (Daniel *et al.*, 1967; Paton, 1968a). Rabbit aortic strips continue to respond to adrenaline, K^+ and angiotensin during anoxia provided that D-glucose is present (Shibata & Briggs, 1967).

The transient contraction of the detrusor muscle observed immediately after addition of DNP may be related to its ability to depolarize cell membranes (Marshall & Miller, 1964).

This investigation was supported by Alberta Heart Foundation Grant No. 67-44 and by a Senior Research Fellowship from the Canadian Heart Foundation. The technical assistance of Miss Alice Fryer and Miss Evelyn These is gratefully acknowledged.

REFERENCES

AXELSSON, J. & BÜLBRING, E. (1961). Metabolic factors affecting the electrical activity of intestinal smooth muscle. *J. Physiol., Lond.*, **156**, 344–356.

DANIEL, E. E., CARROLL, P. M., ROBINSON, K. & GRAHAM, J. (1967). Adenine nucleotides, metabolic inhibitors, and ion fluxes in smooth muscle. In *Ureteral Reflux in Children*, ed. Glenn, J. F., pp. 29–56. Washington: Nat. Acad. Sci. and Nat. Res. Council.

MARSHALL, J. M. & MILLER, M. D. (1964). Effects of metabolic inhibitors on the rat uterus and on its response to oxytocin. *Am. J. Physiol.*, **206**, 437–442.

PATON, D. M. (1968a). Cation and metabolic requirements for retention of metaraminol by rat uterine horns. *Br. J. Pharmac. Chemother.*, **33**, 277–286.

PATON, D. M. (1968b). Pharmacology of isolated rabbit detrusor muscle. *Clin. Res.*, **16**, 393.

PATON, D. M. (1968c). Effects of calcium, strontium and magnesium on contractility of isolated rabbit detrusor muscle. *Clin. Res.*, **16**, 348.

PATON, D. M. (1968d). Effects of metabolic inhibitors on contractility of isolated rabbit detrusor muscle. *Proc. Int. Union Physiol. Sci.*, **7**, 340.

ROHNER, T. J., KOMINS, J. I. & SCHOENBERG, H. W. (1967). Utilization of glucose by normal, defunctionalized and denervated bladder muscle. *Invest. Urol.*, **5**, 12–19.

SHIBATA, S. & BRIGGS, A. H. (1967). Mechanical activity of vascular smooth muscle under anoxia. *Am. J. Physiol.*, **212**, 981–984.

SLATER, E. C. (1963). Uncouplers and inhibitors of oxidative phosphorylation. In *Metabolic Inhibitors.*, ed. Hochster, R. M. & Quastel, J. H., vol. **2**, pp. 503–516. New York: Academic Press.

URSILLO, R. C. (1961). Electrical activity of the isolated nerve-urinary bladder strip preparation of the rabbit. *Am. J. Physiol.*, **201**, 408–412.

URSILLO, R. C. & CLARK, B. B. (1956). The action of atropine on the urinary bladder of the dog and on the isolated nerve-bladder strip preparation of the rabbit. *J. Pharmac. exp. Ther.*, **118**, 338–347.

WEBB, J. L. (1966). Iodoacetate and iodoacetamide. In *Enzyme and Metabolic Inhibitors*, vol. **3**, pp. 1–284. New York: Academic Press.

(*Received April* 17, 1968)