Metabolic Effects of α - and β -Adrenergic Stimulation of Rat Submaxillary Gland *in vitro*

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(Received 14 July 1976)

1. Incubation of submaxillary-gland slices with isoproterenol, a β -adrenergic agonist, stimulated glucose removal by 41 % and decreased tissue [glucose 6-phosphate] by 50 %. Propranolol blocked these effects of isoproterenol. 2. Phenylephrine, an α -adrenergic agonist, stimulated glucose removal by 35 % and decreased tissue [glucose 6-phosphate] by 75 %. In addition, phenylephrine also completely overcame the inhibition of pyruvate removal caused by acetoacetate metabolism, and decreased tissue [ATP] by 45 %. Phentolamine blocked the effects of phenylephrine. 3. In contrast with β -adrenergic stimulation, α -adrenergic stimulation required exogenous Ca^{2+} . 4. These results explain the different metabolic responses of the submaxillary gland to adrenaline in the presence and absence of exogenous Ca^{2+} .

Adrenaline stimulates glucose utilization and abolishes the inhibitory effect of acetoacetate metabolism on glucose and pyruvate uptake by rat submaxillary-gland slices; these effects are associated with a decrease in tissue [ATP] (Thompson & Williamson, 1975). However, when Ca^{2+} is omitted from the incubation medium, adrenaline no longer causes a decrease in tissue [ATP] or overcomes the inhibitory effect of acetoacetate metabolism on pyruvate uptake (Thompson & Williamson, 1975). The question arises as to why there is this difference in the metabolic responses to adrenaline.

One possibility is that it may be due to differential stimulation of the α - and β -receptors in the different incubation media, since the rat submaxillary gland possesses both α - and β -adrenergic receptors (Emmelin, 1967). Thus the aim of the present work was to determine the metabolic responses that occurred on separate stimulation of the α - and β -receptors by using specific agonists and antagonists, and to determine to what extent the responses depended on exogenous Ca^{2+} .

The results indicate that both α - and β -adrenergic agonists can increase the utilization of glucose by submaxillary-gland slices when Ca^{2+} is present in the incubation medium, but that only β -adrenergic responses can occur in the absence of Ca^{2+} . This suggests that Ca^{2+} is required for the mechanism of action of α -adrenergic agonists.

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Experimental

Materials

Male rats of the Wistar strain, weighing 150–200 g, were used for all experiments.

DL-Isoproterenol hydrochloride [1-(3,4-dihydroxyphenyl)-2-isopropylaminoethanol hydrochloride] was obtained from Sigma (London) Chemical Co. Ltd., Kingston-upon-Thames, Surrey, U.K. Phenylephrine [(–)-1-(*m*-hydroxyphenyl)-2-methylaminoethanol] for injection (1 %, w/v in distilled water) was obtained from The Boots Co. Ltd., Nottingham, U.K. Propranolol hydrochloride [1-isopropylamino-3-(naphth-1-yl-oxy)propan-2-ol hydrochloride] was obtained from I.C.I. Ltd., Pharmaceutical Division, Macclesfield, Cheshire, U.K., and phentolamine mesylate [2-(*N-m*-hydroxyphenyl-*p*-toluidinomethyl)-2-imidazoline methanesulphonate] was obtained from Ciba Laboratories Ltd., Horsham, Sussex, U.K. Enzymes, coenzymes and sodium pyruvate were obtained from Boehringer Corp. (London) Ltd., London W.5, U.K. Glucose and all other chemicals were obtained from BDH Chemicals Ltd., Poole, Dorset, U.K. Sodium acetoacetate was prepared from ethyl acetoacetate as described by Krebs & Eggleston (1945).

Methods

The rats were killed by cervical dislocation and submaxillary-gland slices prepared as previously described (Thompson & Williamson, 1975).

Tissue slices (200 ± 10 mg wet wt.) were incubated

in 50ml Erlenmeyer flasks with Krebs-Henseleit (1932) bicarbonate saline (5ml) containing glucose (5mm) and appropriate additions, and gassed with $O_2 + CO_2$ (95:5), then sealed with rubber stoppers. Some incubations were carried out in bicarbonate saline from which Ca^{2+} had been omitted ($CaCl_2$ being replaced by an equivalent amount of $NaCl$). The flasks were incubated for 1h at 37°C in a shaking water bath.

At the end of the incubation period the slices were quickly removed from the medium, blotted on Whatman no. 1 filter paper and immediately dropped into beakers containing liquid N_2 . The frozen slices were ground to a powder with a cold pestle and mortar, then 6% (w/v) $HClO_4$ (5ml) was added and grinding continued for 2min to extract the metabolites. A sample (4ml) of the medium was acidified with 30% $HClO_4$ (0.4ml). A control flask containing bicarbonate saline and additions but no tissue was treated in the same manner. The tissue extract and acidified medium were centrifuged for 10min at 2000g in the cold and then the supernatant fluid was neutralized as previously described (Thompson & Williamson, 1975). The tissue extract was assayed for glucose 6-phosphate and ATP (Lamprecht & Trautschold, 1963) and the medium for glucose (Slein, 1963), pyruvate and lactate (Hohorst *et al.*, 1959).

Glucose removal was calculated from the difference between the glucose present in the control and that remaining in the experimental flask after incubation.

Results

Effects of isoproterenol, a β -adrenergic agonist

The addition of isoproterenol (10 μ g/ml) to the incubation medium stimulated glucose removal by submaxillary-gland slices by approx. 40% (Table 1). This was accompanied by a 50% decrease in tissue [glucose 6-phosphate], which suggested that isoproterenol had stimulated the phosphofructokinase step. However, isoproterenol had no effect on tissue [ATP]. In the present experiments the inhibition of glucose utilization by acetoacetate was less marked than previously reported (Thompson & Williamson, 1975) and was not statistically significant. Isoproterenol overcame the decreased uptake of glucose that occurred when acetoacetate was also present in the incubation medium, although the block at the pyruvate dehydrogenase step caused by acetoacetate (see Thompson & Williamson, 1975) was still apparent because the lactate+pyruvate pool was about 27% higher than with glucose and isoproterenol (Table 1). In agreement with this observation was the finding that isoproterenol was unable to overcome the inhibitory effect of acetoacetate metabolism on pyruvate uptake (Table 2). These results can be explained if isoproterenol can only stimulate the

Table 1. *Effects of isoproterenol, a β -adrenergic agonist, on glucose metabolism and some metabolite concentrations in rat submaxillary-gland slices*

Submaxillary-gland slices (200mg wet wt.) were incubated for 1h at 37°C in Krebs-Henseleit (1932) bicarbonate saline (5ml) gassed with $O_2 + CO_2$ (95:5), and with appropriate additions: glucose (5mm), acetoacetate (2.5mm), isoproterenol (10 μ g/ml) and propranolol (20 μ g/ml). Determinations were carried out as described under 'Methods'. The results are expressed in μ mol/h per g wet wt. $\pm$ s.d. or μ mol/g wet wt. $\pm$ s.d., with the numbers of experiments indicated in parentheses. Values that are statistically different by Student's <i>t</i> test from the appropriate glucose and glucose+acetoacetate controls are indicated by: * $P < 0.05$; ** $P < 0.005$.					
Additions	Glucose removal	Lactate formation	Pyruvate formation	Tissue [glucose 6-phosphate]	Tissue [ATP]
Glucose	21.7 $\pm$ 3.9 (10)	8.6 $\pm$ 1.2 (10)	1.16 $\pm$ 0.17 (10)	0.14 $\pm$ 0.02 (8)	0.92 $\pm$ 0.13 (8)
Glucose+isoproterenol	30.7 $\pm$ 4.3 (8)**	16.1 $\pm$ 3.3 (8)**	2.09 $\pm$ 0.57 (8)**	0.07 $\pm$ 0.02 (8)**	0.82 $\pm$ 0.12 (8)
Glucose+isoproterenol+propranolol	21.6 $\pm$ 4.9 (7)	10.4 $\pm$ 1.6 (5)	0.94 $\pm$ 0.42 (7)	0.11 $\pm$ 0.02 (4)	0.83 $\pm$ 0.12 (4)
Glucose+acetoacetate	19.0 $\pm$ 5.2 (9)	11.3 $\pm$ 2.2 (9)	1.69 $\pm$ 0.27 (9)		
Glucose+acetoacetate+isoproterenol	26.0 $\pm$ 6.2 (8)*	19.5 $\pm$ 3.2 (8)**	3.73 $\pm$ 1.03 (8)**		
Glucose+acetoacetate+isoproterenol+propranolol	19.7 $\pm$ 2.6 (4)	13.9 $\pm$ 4.7 (4)	1.94 $\pm$ 0.50 (4)		

Table 2. Ability of α - and β -adrenergic agonists to overcome the inhibition of pyruvate removal by acetoacetate metabolism in submaxillary-gland slices

Submaxillary-gland slices (200mg wet wt.) were incubated for 1 h at 37°C in Krebs–Henseleit bicarbonate saline (5 ml) gassed with O₂+CO₂ (95:5), and with appropriate additions: pyruvate (2mM), acetoacetate (2.5mM), isoproterenol (10 μ g/ml) and phenylephrine (10 μ g/ml). The values are expressed as μ mol/h per g wet wt. $\pm$ s.d. or as μ mol/g wet wt. $\pm$ s.d., with the numbers of experiments indicated in parentheses. Values that show a significant reversal of acetoacetate inhibition by Student's *t* test are indicated by: * *P* < 0.005.

Additions	Pyruvate removal	Lactate formation	Tissue [ATP]
Pyruvate	34.7 $\pm$ 3.8 (6)	9.5 $\pm$ 1.8 (4)	0.84 $\pm$ 0.14 (6)
Pyruvate+acetoacetate	27.6 $\pm$ 2.2 (6)	13.5 $\pm$ 1.1 (4)	0.92 $\pm$ 0.14 (6)
Pyruvate+acetoacetate+isoproterenol	29.6 $\pm$ 2.8 (4)	13.9 $\pm$ 0.6 (4)	0.83 $\pm$ 0.06 (4)
Pyruvate+acetoacetate+phenylephrine	34.8 $\pm$ 1.7 (4)*	13.0 $\pm$ 1.5 (4)	0.53 $\pm$ 0.02 (4)*

Table 3. Effects of isoproterenol on glucose metabolism in submaxillary-gland slices incubated in a Ca²⁺-depleted medium

Submaxillary-gland slices (200mg wet wt.) were incubated for 1 h at 37°C in Krebs–Henseleit (1932) bicarbonate saline (5 ml) from which Ca²⁺ had been omitted (CaCl₂ replaced by an equivalent amount of NaCl), gassed with O₂+CO₂ (95:5), and with appropriate additions: glucose (5mM), acetoacetate (2.5mM) and isoproterenol (10 μ g/ml). The results are the average of two experiments and are expressed as μ mol/h per g wet wt., with the ranges in parentheses.

Additions	Glucose removal	Lactate formation	Pyruvate formation
Glucose	18.6 (18.0–19.2)	8.6 (8.4–8.8)	1.16 (1.09–1.23)
Glucose+isoproterenol	26.2 (25.9–26.5)	18.6 (18.2–19.0)	1.61 (1.52–1.70)
Glucose+acetoacetate	12.4 (12.2–12.6)	10.2 (9.9–10.5)	1.23 (1.17–1.29)
Glucose+acetoacetate+isoproterenol	24.5 (24.0–25.0)	17.6 (16.4–18.8)	2.93 (2.76–3.10)

glycolytic pathway and not entry of carbon into the tricarboxylic acid cycle.

Results analogous to these have been obtained on stimulation of submaxillary-gland slices by adrenaline in a Ca²⁺-depleted medium (Thompson & Williamson, 1975). This observation suggested that the response to β -adrenergic stimulation might not require the presence of exogenous Ca²⁺. Indeed, the stimulatory effects of isoproterenol on glucose metabolism also occurred when Ca²⁺ was absent from the incubation medium (Table 3).

The specific β -antagonist, propranolol (20 μ g/ml), prevented the effects of isoproterenol on the aspects of submaxillary-gland metabolism studied (Table 1), which confirms that isoproterenol is acting via the mediation of β -adrenergic receptors.

Effects of phenylephrine, an α -adrenergic agonist

Addition of phenylephrine (10 μ g/ml) to the incubation medium stimulated glucose removal by submaxillary-gland slices by 35% (Table 4). The decrease in tissue [glucose 6-phosphate] suggested that phenylephrine, like isoproterenol, was acting by causing stimulation at the phosphofructokinase step. But in contrast with the effects of isoproterenol, phenylephrine also appeared to stimulate entry of glucose carbon into the tricarboxylic acid cycle; phenylephrine decreased the amount of pyruvate formed (Table 4) and completely overcame the inhibition of pyruvate

removal caused by the presence of acetoacetate (Table 2). The effects of phenylephrine were accompanied by a 45% decrease in tissue [ATP] (Table 4).

These latter effects are analogous to those responses that occur on stimulation by adrenaline when Ca²⁺ is present in the medium (Thompson & Williamson, 1975), which suggests that the action of phenylephrine depends on Ca²⁺. Indeed, when Ca²⁺ was omitted from the incubation medium, the metabolic responses to phenylephrine no longer occurred (Table 4).

The responses to phenylephrine were characteristic of those caused by stimulation of α -adrenergic receptors, since they were prevented by phentolamine (20 μ g/ml), a specific α -adrenergic antagonist (Table 4).

Effects of α - and β -adrenergic antagonists on adrenaline action

Since both α - and β -adrenergic agonists stimulated glucose removal (Tables 1 and 4), it would be expected that both α - and β -adrenergic antagonists would need to be present simultaneously to prevent the stimulation of glucose removal brought about by adrenaline (an agonist which can interact with both α - and β -adrenergic receptors). This was indeed found to be the case (Table 5).

Discussion

The metabolic responses resulting from separate

Table 4. *Effects of phenylephrine, an α -adrenergic agonist, on glucose metabolism and some metabolite concentrations in rat submaxillary-gland slices*

Submaxillary-gland slices (200 mg wet wt.) were incubated for 1 h at 37°C in Krebs-Henseleit saline (5 ml) gassed with O₂ + CO₂ (95:5), and with appropriate additions: glucose (5 mM), acetoacetate (2.5 mM), phenylephrine (10 μ g/ml), and phentolamine (20 μ g/ml). Three incubations as indicated were carried out in Krebs-Henseleit saline from which Ca²⁺ had been omitted. Metabolite determinations were carried out as described under 'Methods'. The values are expressed as μ mol/h per g wet wt. $\pm$ s.d. or as μ mol/g wet wt. $\pm$ s.d., with the numbers of experiments indicated in parentheses. Values which are statistically different from the glucose control by Student's *t* test are indicated by: * *P* < 0.001.

Additions	Glucose removal	Lactate formation	Pyruvate formation	Tissue [glucose 6-phosphate]	Tissue [ATP]
Glucose	21.3 $\pm$ 2.4 (8)	8.85 $\pm$ 1.6 (10)	1.42 $\pm$ 1.5 (10)	0.12 $\pm$ 0.02 (8)	0.85 $\pm$ 0.09 (8)
Glucose + phenylephrine	28.9 $\pm$ 3.3 (8)*	13.3 $\pm$ 1.7 (7)*	0.98 $\pm$ 0.11 (8)*	0.03 $\pm$ 0.01 (6)*	0.47 $\pm$ 0.06 (8)*
Glucose + phenylephrine + phentolamine	21.3 (2)	8.06 (2)	1.24 (2)	0.13 (2)	0.96 (2)
Glucose + phenylephrine (Ca ²⁺ omitted)	22.5 $\pm$ 3.4 (3)	11.2 $\pm$ 1.3 (3)	1.46 $\pm$ 0.08 (3)	0.09 $\pm$ 0.01 (3)	0.72 $\pm$ 0.10 (3)

Table 5. *Effect of α - and β -adrenergic antagonists on the stimulation of glucose removal by adrenaline in rat submaxillary-gland slices*

Submaxillary-gland slices (200 mg wet wt.) were incubated for 1 h at 37°C in Krebs-Henseleit (1932) bicarbonate saline (5 ml) gassed with O₂ + CO₂ (95:5), and with appropriate additions: glucose (5 mM), adrenaline (10 μ g/ml), propranolol (20 μ g/ml) and phentolamine (20 μ g/ml). Glucose removal was determined as described under 'Methods'. The values are expressed as μ mol/h per g wet wt. $\pm$ s.d. for the numbers of experiments indicated in parentheses. The value that is statistically different from the glucose + adrenaline control by Student's *t* test is indicated by: * *P* < 0.02.

Additions	Glucose removal
Glucose	20.7 $\pm$ 1.7 (5)
Glucose + adrenaline	25.5 $\pm$ 1.7 (3)
Glucose + adrenaline + propranolol	26.5 $\pm$ 2.6 (3)
Glucose + adrenaline + phentolamine	26.6 $\pm$ 3.2 (3)
Glucose + adrenaline + propranolol + phentolamine	20.5 $\pm$ 1.5 (4)*

stimulation of the α - and β -adrenergic receptors of rat submaxillary gland have not been studied. β -Adrenergic stimulation, as characterized by the response to isoproterenol, causes an increase in glucose removal; one site of action seems to be at the phosphofructokinase step (as indicated by a decrease in [glucose 6-phosphate]), but the stimulation does not appear to be due to allosteric regulation of this enzyme by ATP, since [ATP] is unaffected by isoproterenol. These responses do not depend on the presence of Ca²⁺ in the incubation medium.

On the other hand, α -adrenergic stimulation, as characterized by the response to phenylephrine, in addition to increasing glucose uptake, also overcomes the inhibition of pyruvate uptake by acetoacetate metabolism. This appears to be the result of stimulation of the pyruvate dehydrogenase step as well as of the phosphofructokinase step, and is associated with a decrease in tissue [ATP]. The presence of Ca²⁺ in the incubation medium is necessary for these responses.

These results therefore explain why the metabolic responses to adrenaline in the presence of exogenous Ca²⁺ are different from those observed in the absence of Ca²⁺ (Thompson & Williamson, 1975). Adrenaline is able to stimulate both the α - and β -adrenergic receptors, but in a Ca²⁺-depleted medium only those responses resulting from stimulation of the β -adrenergic receptor are observed, whereas in the complete medium the responses to α -adrenergic receptor stimulation can also occur, namely stimulation of the pyruvate dehydrogenase step and a decrease in [ATP].

Different responses to α - and β -adrenergic stimulation also occur in the rat parotid gland. From studies

with rat parotid slices (Batzri & Selinger, 1973; Batzri *et al.*, 1973) and isolated parotid acinar cells (Mangos *et al.*, 1975) it appears that α -adrenergic stimulation causes K^+ efflux, whereas β -adrenergic stimulation causes protein secretion. As yet the possibility has not been ruled out that the different metabolic responses to α - and β -adrenergic stimulation in the submaxillary gland may be the result of stimulation of these different secretory processes with their associated demands for energy.

A metabolic response in rat parotid fragments which appears to be specifically mediated by α -adrenergic stimulation is the stimulation of phosphatidylinositol metabolism (Michell & Jones, 1974; Oron *et al.*, 1973). However, this stimulation does not appear to require Ca^{2+} , nor is it promoted by the ionophore A23187 (Michell, 1975).

The present results also raise the question of the role Ca^{2+} plays in the mechanism of action of α -adrenergic agonists, whether for hormone-receptor interaction or for transmitting the signal from the membrane into the cell. The calcium ionophore A23187 has been found to mimic some of the effects of adrenaline on the metabolism of rat submaxillary gland (Thompson & Williamson, 1976), namely those that require exogenous Ca^{2+} . The present results show that these effects are the same as those brought about by α -adrenergic stimulation. Since ionophore A23187 appears to mimic the effects of α -adrenergic stimulation, it seems reasonable to conclude that the α -adrenergic responses may result from changes in the movement of Ca^{2+} into the cell or between intracellular compartments. In analogous experiments, Herman & Rossignol (1975) have concluded that the β -adrenergic effect of noradrenaline on glycogenolysis in submaxillary gland is mediated by cyclic AMP, whereas the α -adrenergic and cholinergic effects on this process are dependent on Ca^{2+} influx into the cell. Still to be explored is the possibility that changes in [cyclic GMP] may also be involved in the mechanism of α -adrenergic stimulation. β -Adrenergic responses, on the other hand, are generally mediated via activation of the membrane-bound adenylate cyclase, which increases [cyclic AMP]

(Robison *et al.*, 1971). The submaxillary-gland-slice preparation may prove to be a useful model system for studying the interrelationships between the different mediators of α - and β -adrenergic stimulation.

This work was supported by grants from the Medical Research Council and the U.S. Public Health Service (Grant no. AM-11748). M. P. T. was a holder of an 1851 Overseas Science Scholarship. D. H. W. is a member of the External Staff of the Medical Research Council.

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