THE ABILITY OF THE CALCIUM IONOPHORE A-23187 TO MIMIC SOME OF THE EFFECTS OF ADRENALINE ON THE METABOLISM OF RAT SUBMAXILLARY GLAND

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1. Introduction

Exogenous Ca^{2+} is required for carbamylcholine to stimulate glycogenolysis [1] and for noradrenaline to stimulate glucose oxidation [2] in rat submaxillary gland slices. It is also required for adrenaline to completely overcome the inhibition of glucose utilization caused by acetoacetate [3]. This latter effect of adrenaline is associated with a decrease of tissue [ATP] which occurs only when exogenous Ca^{2+} is present [3]. These findings suggest that Ca^{2+} might be involved in the mechanism by which adrenaline exerts some of its metabolic effects.

Ca^{2+} may simply be required for the hormone to bind to its receptor in the membrane or the hormone-receptor interaction may stimulate movement of Ca^{2+} into the cell and thus initiate the metabolic response. The ionophore A-23187 is capable of facilitating the movement of Ca^{2+} and other divalent cations across biological membranes [4] and can be used as a tool to investigate whether changes in Ca^{2+} flux can alter cellular metabolism.

Indeed A-23187 stimulates glycogen breakdown in submaxillary gland slices [5] and the results reported here show that A-23187 can also stimulate glucose uptake and cause the tissue [ATP] to decrease. On the other hand, adrenaline was no longer able to stimulate glucose metabolism in the presence of tetracaine or Ni^{2+}, which appear to inhibit Ca^{2+} flux into cells.

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2. Materials and methods

Male rats of the Wistar strain, weighing 150–200 g were used for all experiments. A-23187 was a gift from Eli-Lilly & Co. (Indianapolis, Ind., USA). Amethocaine hydrochloride (tetracaine) was obtained from Glaxo Laboratories Ltd., Greenford, UK. All other enzymes, coenzymes, were obtained as previously described [3].

A stock solution of A-23187 was prepared in methanol at a concentration of 1 mg/ml. Appropriate additions of this solution were made to the incubation media to give a final concentration of 10 μg/ml (less than 0.1% methanol). Incubations carried out with addition of methanol (0.1%) showed no significant differences from the control for the parameters measured.

The rats were killed by cervical dislocation and the submaxillary glands dissected out into ice-cold saline. Slices (0.3–0.4 mm thick) were cut with a razor blade.

Incubations were carried out in 50 ml Erlenmeyer flasks. Each flask contained 200 ± 10 mg wet wt. slices in Krebs–Henseleit [6] bicarbonate saline (5 ml) gassed with 95% O_2, 5% CO_2, and with glucose (5 mM) and appropriate additions, and was incubated for 1 h at 37°C in a Dubnoff-type shaker.

At the end of the incubation period the slices were quickly removed from the medium, blotted on Whatman No.1 filter paper, then immediately dropped into beakers containing liquid N_2. The frozen slices were powdered with a cold pestle and mortar, then 5% $HClO_4$ (5 ml) was added and grinding continued for 2 min to extract the metabolites. A sample of the medium (4 ml) was acidified with 30% $HClO_4$ (0.4 ml).

A control flask containing saline plus additions but no tissue was treated in the same manner. The tissue extracts and acidified medium were centrifuged for 10 min at 2000 *g* in the cold and then the supernatants neutralized. Glucose 6-phosphate and ATP [7] were determined on the tissue extract and glucose [8] and lactate [9] were determined on the medium.

3. Results

The addition of A-23187 (10 μg/ml) to the incubation medium caused a 27% stimulation of glucose removal by submaxillary gland slices and overcame the inhibition of glucose uptake caused by acetoacetate (2.5 mM; table 1). This stimulation of glucose removal appeared to be due to activation at the phosphofructokinase reaction, as [glucose 6-phosphate] was decreased compared with the control (table 1). A-23187 caused a 40% decrease in the tissue [ATP] (table 1); this decrease could possibly explain the observed stimulation of the phosphofructokinase reaction (for discussion see [3]). A greater proportion of the glucose removed could be accounted for as lactate when A-23187 was present. None of these effects was observed when Ca^{2+} was omitted from the incubation medium (table 1), which suggests that they result from enhancement of the flux of Ca^{2+} into the cell.

Stimulation of glucose utilization with an accompanying decrease in the tissue [ATP] also occurs when adrenaline is added to the incubation medium (table 2 and [3]). So if this indeed was mediated by

Table 1
Effects of ionophore A-23187 on glucose metabolism, [glucose 6-phosphate] and [ATP] in submaxillary gland slices

Additions	Glucose removal	Lactate formed	Tissue [glucose 6-phosphate]	Tissue [ATP]
Glucose	21.4 ± 2.4 (8)	8.2 ± 0.5 (8)	0.14 ± 0.03 (6)	0.83 ± 0.07 (5)
Glucose + A-23187	27.1 ± 3.5 (8)*	14.9 ± 2.9 (8)*	0.06 ± 0.01 (7)*	0.48 ± 0.14 (6)*
Glucose + A-23187 (Ca^{2+} omitted)	19.9 ± 1.6 (8)	9.9 ± 1.1 (9)	0.14 ± 0.02 (4)	0.84 ± 0.11 (4)
Glucose + acetoacetate	17.0 ± 3.6 (8)	11.9 ± 2.1 (4)	0.19 ± 0.02 (5)*	0.88 ± 0.14 (6)
Glucose + acetoacetate + A-23187	26.2 ± 3.9 (6)*	24.0 ± 2.1 (4)*	0.05 ± 0.01 (6)*	0.40 ± 0.09 (6)*

Metabolite changes are mean values (± S.D.) expressed as μmoles/h/g wet wt. and tissue concentrations are mean values (± S.D.) expressed as μmoles/g wet wt. Values which are statistically different from the appropriate control by the Student's t test are indicated by * : $P < 0.005$.

Table 2
Effects of tetracaine on glucose metabolism, [glucose 6-phosphate] and [ATP] in submaxillary gland slices

Additions	Glucose removal	Lactate formed	Tissue [glucose 6-phosphate]	Tissue [ATP]
Glucose	20.1 ± 3.2 (8)	9.4 ± 0.84 (5)	0.14 ± 0.02 (8)	0.95 ± 0.11 (8)
Glucose + tetracaine	24.4 ± 1.2 (4)	15.3 ± 1.4 (4)*	0.09 ± 0.01 (4)*	0.87 ± 0.13 (4)
Glucose + adrenaline	31.4 ± 3.1 (4)*	14.6 ± 0.8 (4)*	0.04 ± 0.02 (5)*	0.64 ± 0.06 (5)*
Glucose + adrenaline + tetracaine	23.5 ± 3.2 (4)	18.2 ± 2.3 (5)*	0.08 ± 0.04 (5)*	0.61 ± 0.21 (5)*

Units as for table 1. Values that are statistically different by the Student's t test from the glucose control are indicated by * : $P < 0.005$.

Table 3
Effects of nickel chloride on glucose metabolism, [glucose 6-phosphate] and [ATP] in submaxillary gland slices

Additions	Glucose removal	Lactate formed	Tissue [glucose 6-phosphate]	Tissue [ATP]
Glucose	19.2 ± 5.5 (6)	8.4 ± 1.5 (6)	0.12 (2)	0.86 ± 0.06 (4)
Glucose + nickel chloride	18.9 ± 4.6 (5)	8.4 ± 1.5 (6)	0.19 (2)	0.85 ± 0.08 (4)
Glucose + adrenaline	31.7 ± 2.1 (4)*	13.4 ± 2.7 (4)*	0.02 (2)	0.46 ± 0.03 (4)*
Glucose + adrenaline + nickel chloride	16.1 ± 3.3 (6)	7.9 ± 1.0 (6)	0.16 (2)	0.76 ± 0.05 (4)

Units as for table 1. Values which are statistically different by the Student's t test from the glucose control are indicated by * : $P < 0.005$.

an enhanced flux of Ca^{2+} into the cell, then the effects of adrenaline should be prevented by agents which inhibit this process. Tetracaine (1 mM) is a compound capable of altering the movement of Ca^{2+} across membranes [10] and it prevented the stimulation of glucose removal by adrenaline (10 μg/ml) (table 2). The decrease in tissue [glucose 6-phosphate] was also partially prevented by tetracaine (table 2), but the lowering of the tissue [ATP] was unaffected. An indication that tetracaine may have other metabolic effects is the greater accumulation of lactate in its presence (table 2).

An ability to interfere with normal calcium flux has been attributed to Ni^{2+} [11]. In submaxillary gland slices the addition of nickel chloride (0.5 mM) prevented the stimulation of glucose uptake and the accompanying decreases in tissue [glucose 6-phosphate] and [ATP] caused by adrenaline (table 3).

4. Discussion

Since A-23187 is known to facilitate the movement of Ca^{2+} across biological membranes [4] it appears from the present results that changes in Ca^{2+} movement and/or changes in Ca^{2+} concentration within the submaxillary gland cells may be sufficient to bring about changes in glucose metabolism. It this is true then the trigger mechanism for some of the metabolic responses to adrenergic stimulation in submaxillary gland, especially those requiring exogenous Ca^{2+}, may consist of a shift of Ca^{2+} from the cell environment (exogenous Ca^{2+}) and/or from intracellular sites (possibly from the mitochondria, secretory granules or other compartments) towards special zones in the cytoplasm where the response occurs. The fact that both tetracaine and Ni^{2+}, which interfere with membrane Ca^{2+} channels, prevent the stimulation of glucose utilization by adrenaline supports this suggestion.

The metabolic responses to adrenaline that require Ca^{2+} may be the result of stimulation of α-adrenergic receptors since it is known that adrenergic stimulation via the α-receptor in a number of other tissues [12] including the parotid gland [13] requires exogenous Ca^{2+}. In contrast, it is generally accepted that changes in [cyclic AMP] mediate the responses to β-adrenergic stimulation [14] and it is therefore significant that exogenous cyclic AMP is unable to stimulate glucose utilization or decrease [ATP] in the system described here [3]. Phenylephrine, a specific α-adrenergic agonist, requires exogenous Ca^{2+} in order to bring about its effects on submaxillary gland, whereas isoproterenol, a specific β-adrenergic agonist, does not (M.P. Thompson and D.H Williamson, unpublished experiments). Similarly, Herman and Rossignol [1] 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.

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