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Buffer-Dependent Actions of Insulin and Halothane on Glucose Metabolism in Isolated Rat Atria[1] (38059)

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A large number of experiments in isolated tissue preparations have suggested that the glycolytic pathway is buffer-dependent. Either bicarbonate or Tris must be present as the buffer in the extracellular medium for optimal glycolytic activity. In the absence of buffer or with phosphate buffered medium, glycolysis is inhibited. Functional studies in rat atria have shown that glucose is more effective for maintenance of the force of contraction in Tris or bicarbonate containing medium than in phosphate or unbuffered medium (1). Fructose and glucose were ineffective as energy sources for contractile activity in bicarbonate-free phosphate buffered medium whereas pyruvate was effective (2). With bicarbonate present all three potential substrates were effective (3). In rat ventricle strips suspended in 1 m*M* phosphate medium, Berman and coworkers (4, 5) showed that in contrast to strips bathed in bicarbonate buffer, pyruvate or acetate were more effective substrates for the support of contractile activity than was glucose. Shaw and Stadie (6, 7) reported that in rat diaphragm muscle incubated in 50 m*M* phosphate buffered medium phosphofructokinase was inactive unless 25 m*M* bicarbonate was added to the medium.

The effect of insulin on glucose metabolism has been shown to be affected by the buffer. Lactic acid production from glucose in isolated rat diaphragm was increased by insulin in bicarbonate–phosphate medium but was unaffected if bicarbonate was omitted. Glycogen production from glucose, however, was stimulated by insulin whether bicarbonate was present or not (6).

Studies concerned with the action of inhalation anesthetics on carbohydrate metabolism in isolated tissues have been performed in media buffered with phosphate (8, 9), bicarbonate (10–16) and Tris (17, 18). To our knowledge, no comparative study has yet been performed to determine whether the nature of the buffer influences the effect of the anesthetic.

The purposes of the present investigation are: (1) to extend the above observations by comparing glucose metabolism of isolated rat atria in Tris and in phosphate buffered medium. (2) to determine whether the nature of the buffer influences the stimulatory action of insulin and the inhibitory action of the inhalation anesthetic, halothane, on glucose metabolism.

Methods. Atria from fed male rats weighing between 225 and 325 g were incubated at 30°C in 2 ml of medium having the following composition (m*M*): NaCl, 120; KCl, 6; $MgSO_4 \cdot 7H_2O$, 1.34; NaH_2PO_4, 1.21; $CaCl_2 \cdot 2H_2O$, 1.22; glucose 5.56; and either Tris (tris[hydroxymethyl]aminomethane), 25.3 or NaH_2PO_4, 25.3. In addition, 0.2 μCi of [6-^{14}C] glucose (Amersham/Searle Corp.) was added to each flask. The medium in which the tissue was incubated was bubbled with 100% O_2 prior to the start of a 1 hr incubation. CO_2 was liberated from the me-

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TABLE I. Effects of Insulin (100 mU/ml) and Halothane (1 m*M*) on CO_2 Production from Glucose in Tris or Phosphate Buffered Media.

	nmoles glucose/g dry wt/hr oxidized to CO_2	
	Tris	Phosphate
Control	680 ± 28 (94)	300 ± 19 (24)
Insulin	917 ± 24 (7) *	318 ± 29 (8)
Halothane	380 ± 38 (12) *	280 ± 39 (8)
Insulin + Halothane	660 ± 98 (4) **	

Values are means ± SEM. Figures in parentheses represent number of experiments.
* $P < .001$ compared to control.
** $P < .05$ compared to insulin.

dium with 0.2 ml of 70% $HClO_4$ and trapped with 0.2 ml of 2 *N* KOH. A 0.1 ml aliquot of this KOH was placed in 10 ml of Bray's solution (19) and was counted by liquid scintillation. Appropriate correction factors based on previous studies (20) were used to determine n*M* glucose. Glycogen was isolated and prepared for measurement via alcoholic KOH extraction (21), sulfuric acid hydrolysis and KOH neutralization. Subsequently labeled glucose incorporated into this glycogen was counted via liquid scintillation. Additional details of the methods have been published (17).

Results. The control values for glucose oxidation to CO_2 in atria incubated in phosphate buffered medium were considerably lower than the control values for glucose oxidation in Tris buffer (Table I). Insulin (100 mU/ml) significantly increased CO_2 production from glucose in Tris buffered medium but had no effect in phosphate medium. Halothane (1 m*M*) significantly decreased CO_2 production in Tris buffer whether insulin was present or not but had no effect in phosphate medium.

In contrast to the results with CO_2 production from glucose seen in Table I, there was no difference in glucose incorporation into glycogen seen in control atria incubated in Tris or in phosphate buffered medium (Table II). Insulin (100 mU/ml) stimulated and halothane (1 m*M*) inhibited glucose incorporation into glycogen in either buffer. With insulin present, halothane still inhibited glucose incorporation into glycogen in Tris buffer.

A very high concentration of halothane (8 m*M*), thought to inhibit electron transport (17), inhibited CO_2 production from glucose in both buffers (Table III).

Discussion. The CO_2 production studies indicate a greater utilization of glucose in Tris than in phosphate buffer. This is in agreement with functional studies in which glucose was more effective in supporting contractile activity if Tris, rather than phosphate, was the buffer (1).

The stimulatory action of insulin on glucose catabolism seen with bicarbonate-buffered medium by Shaw and Stadie (6) in isolated rat diaphragm was also seen by us in rat atria using Tris. However, insulin did

TABLE II. Effects of Insulin (100 mU/ml) and Halothane (1 m*M*) on Glucose Incorporation into Glycogen in Tris or Phosphate Buffered Media.

	nmoles glucose/g dry wt/hr incorporated into glycogen	
	Tris	Phosphate
Control	2517 ± 232 (20)	2583 ± 208 (16)
Insulin	6871 ± 322 (12) *	5966 ± 455 (8) *
Halothane	1160 ± 113 (12) *	1566 ± 247 (8) ***
Insulin + Halothane	2873 ± 573 (4) ****	

Values are means ± SEM. Figures in parentheses represent number of experiments.
*** $P < .01$ compared to control.
* $P < .001$ compared to control.
**** $P < .001$ compared to insulin.

TABLE III. Effect of Halothane (8 m*M*) on CO_2 Production from Glucose in Tris or Phosphate Buffered Media.

	nmoles glucose/g dry wt/hr oxidized to CO_2	
	Tris	Phosphate
Control	680 ± 28 (94)	300 ± 12 (24)
Halothane	323 ± 64 (8) *	167 ± 18 (7) *

Values are means ± SEM. Figures in parentheses represent number of experiments.

* $P < .001$ compared to control.

not have this effect in both diaphragm (6, 7) and atrial preparations (Table I) in phosphate buffer.

Functional studies with bicarbonate in rat and human atria (10–14, 16) and metabolic studies with Tris in rat atria and cerebral cortex slices (17, 18) have demonstrated a halothane-induced block in glycolysis at a site prior to the phosphofructokinase step. However, such a block cannot be demonstrated in phosphate only buffer (Table I). In order to inhibit CO_2 production from glucose in phosphate buffer, very high concentrations of halothane were required (Table III). Such concentrations probably act at a site in the electron transport system (17).

In contrast to the CO_2 production studies, glucose was converted into glycogen equally well in Tris or in phosphate buffer. In addition, insulin stimulated and halothane inhibited glucose incorporation into glycogen in either buffer. Shaw and Stadie found insulin's stimulatory action on glycogen production from glucose to be present in rat diaphragm with either bicarbonate plus phosphate or phosphate alone in the medium (6, 7).

If we assume glucose-6-phosphate to be an obligatory intermediate for the conversion of glucose to glycogen and CO_2, the site of the inhibitory action of phosphate buffered medium on CO_2 production from glucose must be below glucose-6-phosphate in glycolysis. Glucose uptake and phosphorylation can be ruled out as the site of inhibition when phosphate is the only buffer, since both sites are involved in the conversion of glucose to glycogen or to CO_2 and only glucose oxidation to CO_2 was inhibited by phosphate.

The mechanism of inhibition probably results from the absence of an adequate buffer (Tris or bicarbonate) rather than the presence of phosphate. Ko *et al.* (1) showed a functional inhibition of glucose utilization in rat atria with no buffer present or with phosphate as the only buffer. When Tris or bicarbonate, as well as phosphate, were present however, glucose was utilized for contractile force. Shaw and Stadie (6, 7) presented evidence for an inhibition of glycolysis at the phosphofructokinase (PFK) step with phosphate as the only buffer. With both bicarbonate and phosphate present the PFK step became active.

A block at the PFK step with phosphate as the only buffer might explain the prevention of the stimulatory effects of insulin and inhibitory effects of halothane in phosphate buffered medium since both insulin and halothane are thought to act at, or prior to, the PFK step (10–14, 16–18, 22). We conclude that phosphate is an inappropriate buffer for rat atrial studies dealing with the effects of agents like insulin and halothane which are thought to act at an early site in glycolysis.

Summary. Rat atria incubated in 25 m*M* phosphate buffered medium converted glucose to CO_2 at a rate less than half that seen in 25 m*M* Tris. The stimulatory action of 100 mU/ml insulin and the inhibitory action of 1 m*M* halothane on CO_2 production from glucose seen in Tris medium did not occur in phosphate buffered medium. A higher concentration of halothane (8 m*M*) was required to inhibit CO_2 production from glucose in phosphate buffer. In contrast, the rate of incorporation of glucose into glycogen was similar in phosphate and Tris buffered medium. This glucose incorporation is stimulated by insulin and inhibited by halothane in both buffer systems. We conclude that with phosphate as the only buffer glycolysis is inhibited at some site subsequent to the production of G-6-P from glucose, probably at the phosphofructokinase step, and that this inhibition interferes with the actions of insulin and halothane on glucose catabolism.

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