Glucose Oxidation in Isoproterenol-Stimulated Salivary Glands of the Rat[1] (36067)

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Chronic administration of isoproterenol (IPR) induces hypertrophy of the salivary glands of rats (1) and mice (2). A single injection of this drug results in a marked stimulation of DNA synthesis in rodent salivary glands; maximum synthesis occurring about 24 hr after the drug (3, 4).

Although much attention has been devoted to the study of this effect, relatively little work has been done concerning alterations in carbohydrate metabolism in this organ under the influence of IPR. Malamud and Baserga (5) in a recent report, showed a temporal relationship between glycogen synthesis and initiation of DNA synthesis following administration of IPR. The glycogen that accumulated after IPR treatment, but prior to initiation of DNA synthesis, was thought to serve as a source of energy and pentose, and possibly metabolized via the pentose monophosphate shunt pathway (PMS).

The present work is concerned with an examination of the effect of IPR upon glucose metabolism in the salivary gland of the rat. Of particular interest was an attempt to assess the relative involvement of the Embden-Meyerhoff pathway (EMP) and the PMS pathways in glucose oxidation under the influence of IPR.

Materials and Methods. Male Sprague-Dawley rats (180–200 g) were used in all experiments. Isoproterenol [1-(3,4-dihydroxyphenyl)-2-isopropylaminoethanol hydrochloride], obtained from Sigma Chemical Co., St. Louis, MO was dissolved in water immediately before use. Each animal was given IPR (30 mg/kg of body wt) ip in a volume of 0.25 ml. Methotrexate (2,4-diamino-N^{10}-methyl tetrahydrofolic acid), obtained from Lederle Laboratories, Pearl River, NY was administered ip as a sodium salt solution (0.75 mg/kg of body wt) once in 2 days.

Animals were killed by decapitation; and submaxillary salivary glands (and when necessary, the liver and diaphragm) were quickly dissected free of adhering tissues and placed in chilled modified Krebs–Ringer bicarbonate buffer of the following composition (mM): NaCl, 120; KCl, 5; MgCl, 1; $CaCl_2$, 0.0475; glucose, 5.5; $NaHCO_3$, 25; to give pH 7.4 in the presence of 95% O_2 and 5% CO_2.

Tissue slices (150 mg) prepared with a Stadie-Riggs hand microtome were placed in Erlenmeyer flasks containing 5 ml of the above medium and additional glucose-1-^{14}C or glucose-6-^{14}C (0.2 μCi equiv to 3×10^{-5} mmoles of glucose and giving a total activity of 1.02×10^7 and 1.00×10^7 cpm, respectively) obtained from New England Nuclear (Boston, MA). Following gassing (15 min) with 95% O_2 and 5% CO_2, each flask was stoppered with a rubber stopper carrying a plastic center well for later addition of Hyamine. At the end of 1 hr of incubation at 37°, 0.2 ml of 60% perchloric acid was added to the medium (via a 25 gauge needle inserted through the stopper) to stop all enzymatic reactions and to liberate CO_2. Similarly, Hyamine (0.2 ml) was added to the center well to trap CO_2. The flasks were allowed to stand at room temperature for 1 hr. Center wells were removed and placed in scintillation vials containing 10 ml of Bray's solution. Radioactivity was determined in a Packard Tricarb scintillation counter.

Glucose oxidation is reported as the

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TABLE I. Effect of Chronic Administration of IPR upon Body Weight, Weights of Salivary Glands, Liver, and Kidney.[a]

Group no.	Treatment	Body wt (% Δ)	Submaxillary gland	Liver	Kidney
1	Control (4)	+2.8 ± 0.5	0.769 ± 0.043	11.450 ± 0.383	2.350 ± 0.028
2	IPR (4)	+3.7 ± 3.3	2.280 ± 0.220[b]	12.983 ± 0.619	2.510 ± 0.194
3	IPR + MTXT (4)	+7.1 ± 3.1	0.730 ± 0.094	10.344 ± 0.720	2.194 ± 0.131

[a] Animals were injected with IPR (300 mg/kg of body wt) daily for 10 days. One group of animals was treated also with MTXT (group 3; 0.75 mg/kg of body wt) once in 2 days. All weights (wet) are reported in grams. Number of animals in each group is given in parentheses.
[b] Values are significantly different from control values.

amount of $^{14}CO_2$ (cpm) produced/g of wet tissue/hr. All data are reported as means (± SE) for the number of observations indicated in parentheses or in a legend.

Results. Effect of chronic administration of IPR upon body weight, weights of salivary glands, liver, and kidney. Table I shows that injection of IPR (300 mg/kg of body wt) daily for 10 days, caused a significant increase in the weight of salivary glands (group 2); but treatment with IPR and methotrexate [(MTXT) (0.75 mg/kg of body wt)] during the same period (group 3) resulted in no changes in the weights of the glands. No significant changes were seen in body weights, weights of livers, or kidneys under the influence of IPR or IPR plus methotrexate.

Effect of added insulin upon glucose oxidation in salivary glands, liver, kidney cortex, and diaphragm slices. When salivary gland, liver, and kidney cortex slices were incubated in the presence of added bovine insulin (50 μU/ml), no significant difference was found in $^{14}CO_2$ production from glucose-6-^{14}C compared with $^{14}CO_2$ produced by corresponding slices that were incubated without insulin (Table II). As expected, diaphragm slices incubated in the presence of insulin produced 2.5 times as much $^{14}CO_2$ as their controls.

Effect of fasting upon glucose oxidation in salivary gland slices. Salivary gland slices from fed (normal) and animals fasted for 56 hr were incubated in absence and in the presence of added bovine insulin (50 μU/ml). Slight and insignificant decrease in $^{14}CO_2$ production from glucose was observed in fasted animals compared with fed controls (Table III). The 1-$^{14}CO_2$/6-$^{14}CO_2$ ratio was the same in the two groups. Added insulin did not induce any significant changes in $^{14}CO_2$ production in both groups.

In vitro *effects of IPR and MTXT upon glucose oxidation in salivary gland and liver slices.* Salivary gland and liver slices were incubated in the presence and absence of IPR and methotrexate (20 μg/ml). Results summarized in Table IV indicate that $^{14}CO_2$ production from glucose-1-^{14}C was not affected by the presence in incubation medium of either of the two drugs.

Glucose oxidation in salivary gland and liver slices from animals 10, 18, 25, and 40 hr after a single injection of IPR. Production of $^{14}CO_2$ from glucose-1-^{14}C and glucose-6-^{14}C by salivary gland and liver slices were determined in animals injected with IPR (300 mg/kg of body wt) 10, 18, 25, 40 hr previously (Table V). Significantly low

TABLE II. Effect of Insulin upon Glucose Oxidation in Salivary Gland, Liver, Kidney Cortex, and Diaphragm Slices.[a]

	$^{14}CO_2$ production (cpm/g/hr)	
Insulin:	Nil	+
Salivary gland (3)	16,900 ± 1550	17,900 ± 1100
Liver (3)	4500 ± 300	4250 ± 1050
Kidney cortex (3)	23,000 ± 1900	22,350 ± 2400
Diaphragm (3)	7050 ± 650	17,300 ± 1350[b]

[a] Tissue slices were incubated in media containing glucose-6-^{14}C in the presence or absence of added bovine insulin (50 μU/ml of medium).
[b] Values are significantly higher than these controls.

TABLE III. Effect of Fasting upon Glucose Oxidation in Salivary Gland Slices.[a]

Tracer	Insulin	$^{14}CO_2$ production (cpm/g/hr) Treatment: Fed	Fasted
Glucose-1-^{14}C	Nil	22,950 ± 1750	18,200 ± 1000
	+	24,350 ± 1750	18,745 ± 1050
Glucose-6-^{14}C	Nil	28,600 ± 7500	19,700 ± 2000
	+	25,350 ± 1050	18,900 ± 1200
1-$^{14}CO_2$/6-$^{14}CO_2$	Nil	0.806 ± 0.144	0.928 ± 0.070
	+	0.961 ± 0.123	0.991 ± 0.115

[a] Tissue slices from control (fed) or animals fasted for 56 hr were incubated in the presence or absence of added bovine insulin (50 μU/ml of medium).

$^{14}CO_2$ production was observed in salivary glands at 10 and at 18 hr compared with control animals ($p < .01$). However, no significant changes were noted at 25 and at 40 hr; the 1-$^{14}CO_2$/6-$^{14}CO_2$ ratio remained constant at all times. By contrast, liver slices showed no depression in $^{14}CO_2$ production at 10 or 18 hr although significantly higher oxidation was observed at 40 hr. As in the case of salivary glands slices, no significant changes were seen in the 1-$^{14}CO_2$/6-$^{14}CO_2$ ratio in liver slices. One group of animals (group 6) received IPR daily for 3 days and was sacrificed 24 hr after the last dose. At this time, salivary glands had enlarged significantly (1.22 ± 0.02 g; $N = 3$) compared with those of controls (0.93 ± 0.001; $N = 3$). However, no significant differences were observed with respect to $^{14}CO_2$ production between slices from control and IPR-treated animals.

Discussion. The present data suggest that within 10 days of daily administration of IPR, submaxillary glands, but not liver or kidneys, undergo hypertrophy to 3 times the normal size. Salivary gland hypertrophy was completely prevented if methotrexate was administered during the same period; thus indicating the dependence of this hypertrophy upon nucleic acid synthesis.

Insulin effects on glucose oxidation were observed only with diaphragm muscle and are in keeping with the well-known effects of the hormone upon glucose transport in muscle. Lack of any effect in liver and salivary gland suggests that, in these tissues, glucose transport may not be rate limiting to its subsequent metabolism (6).

Salivary gland slices produced $^{14}CO_2$ from glucose-1-^{14}C to the same extent as they produced from glucose-6-^{14}C (1-$^{14}CO_2$/6-$^{14}CO_2$ = 1). This is taken to mean that glucose oxidation in such a tissue occurs predominantly via the Embden-Meyerhoff pathway and TCA

TABLE IV. *In Vitro* Effects of IPR and MTXT upon Glucose Oxidation in Salivary Gland and Liver Slices.[a]

Tissue	Tracer	$^{14}CO_2$ production (cpm/g/hr) Drug: Control	IPR	MTXT
Salivary gland	Glucose-1-^{14}C	23,350 ± 3000	21,650 ± 1300	25,100 ± 1550
	Glucose-6-^{14}C	15,150 ± 2250	19,400 ± 2050	20,900 ± 1350
	1-$^{14}CO_2$/6-$^{14}CO_2$	1.541 ± 0.182	1.116 ± 0.104	1.201 ± 0.084
Liver	Glucose-1-^{14}C	13,500 ± 1300	14,075 ± 1510	12,850 ± 750
	Glucose-6-^{14}C	5750 ± 550	5550 ± 300	5450 ± 300
	1-$^{14}CO_2$/6-$^{14}CO_2$	2.422 ± 0.283	2.536 ± 0.184	2.385 ± 0.222

[a] Tissue slices were incubated in the presence or absence of IPR or MTXT (20 μg/ml). Values are means (± SE) of 4 observations.

TABLE V. $^{14}CO_2$ Production in Salivary Gland and Liver Slices of Animals Pretreated with IPR for Various Periods.[a]

Group no.	Time (hr) after IPR treatment	$^{14}CO_2$ production (cpm/g/hr)					
		Salivary gland			Liver		
		1-$^{14}CO_2$	6-$^{14}CO_2$	1-$^{14}CO_2$/6-$^{14}CO_2$	1-$^{14}CO_2$	6-$^{14}CO_2$	1-$^{14}CO_2$/6-$^{14}CO_2$
1	Control (10–16)	21,250 ± 950	18,300 ± 1600	0.890 ± 0.080	14,400 ± 1700	4400 ± 400	3.271 ± 0.274
2	10 (4)	11,400 ± 650	9850 ± 150	1.163 ± 0.063	10,100 ± 800	3500 ± 101	2.886 ± 0.264
3	18 (4)	17,500 ± 500	14,750 ± 800	1.194 ± 0.074	10,300 ± 750	3800 ± 800	2.711 ± 0.371
4	25 (3–4)	18,800 ± 350	15,300 ± 1500	1.241 ± 0.163	10,600 ± 200	4900 ± 300	2.164 ± 0.028
5	40 (3)	21,750 ± 700	19,450 ± 350	1.112 ± 0.046	20,610 ± 1400	7900 ±1200	2.609 ± 0.051
6	24+(3)	22,150 ± 400	18,200 ± 600	1.211 ± 0.018	19,800 ± 800	7710 ± 680	2.568 ± 0.039

[a] Animals (groups 2–5) were given a single injection of IPR (300 mg/kg of body wt) and sacrificed 10, 18, 25, or 40 hr later. One group (group 6) received IPR injections daily for 3 days and was sacrificed 24 hr after the last injection. Tissue slices were incubated in the presence of glucose-1-^{14}C and with glucose-6-^{14}C. Number of observations for each group is given in parentheses.

cycle but little or no oxidation occurs via the pentose monophosphate shunt (7). By contrast, liver slices produced relatively more 1-$^{14}CO_2$ than 6-$^{14}CO_2$ from glucose labeled in the first carbon and sixth carbon, respectively (1-$^{14}CO_2$/6-$^{14}CO_2$ > 1) as shown in Tables IV and V. This observation indicates the presence of an active pentose shunt pathway in liver, a phenomenon previously reported by many investigators (8–10).

Isoproterenol and methotrexate, when added to the incubation media, had no influence upon $^{14}CO_2$ production in either salivary gland or liver slices. By contrast, 10 hr after a single *in vivo* injection of IPR, a 50% decrease in glucose-1-^{14}C and glucose-6-^{14}C oxidation in salivary gland slices, but not in the liver, was observed. At 18 hr, glucose oxidation was still significantly depressed in the salivary gland, but a complete return to normal was achieved by 40 hr. In addition, animals treated with IPR for 3 days (a treatment which induced significant gland hypertrophy) produced no significant alteration in glucose oxidation in the gland, although a slight increase in liver activity was seen.

The decrease in glucose oxidation noted after a single IPR injection might possibly be a result of animals not feeding under the influence of the drug. However, this would be unlikely because no such dramatic change occurs in fasted animals (Table III) and no such effect occurs in the liver (Table V). It appears, therefore, to be a specific response in the salivary gland.

Malamud and Baserga (5) showed that in mice given a single injection of IPR, glycogen deposition in salivary glands increases beginning at 5–6 hr, reaching a peak at 18 hr, and thereafter gradually decreases as DNA synthesis begins. This type of response, however, does not occur in liver nor in muscle. It seems likely that the decrease in glucose oxidation observed in the present studies (Table V) may be the result of a shift from glucose oxidation to predominantly glycogen synthesis under the influence of the drug, in preparation for active DNA synthesis.

In Ref. (5), it is postulated that glycogen, accumulated under the influence of IPR, is

possibly subsequently metabolized via the pentose monophosphate shunt to provide a source of energy and pentose for ensuing DNA synthesis. The present studies (Table IV) do not provide evidence for this hypothesis; since no selective increase in glucose-1-^{14}C utilization in the salivary gland occurred at any time during the response to IPR administration. One may speculate, therefore, that glycogen in the salivary gland provides pentose for DNA synthesis by a process other than the pentose shunt, possibly via the transketolase reaction from fructose-6-phosphate (11). Alternatively, the liver and not the salivary gland may be the source of pentose utilized in IPR-stimulated DNA synthesis in the salivary gland, but this is pure conjecture at this time in the absence of actual experimental evidence.

Summary. Administration of isoproterenol (IPR) to rats induced a 2.5-fold increase in submaxillary glands in 10 days, with no changes in the liver and kidney. This hypertrophy was completely prevented when methotrexate was administered concurrently with IPR. Salivary gland slices from fed or fasted animals, unlike diaphragm muscle, produced the same amount of $^{14}CO_2$ from labeled glucose when incubated in the presence, or absence, of insulin. This suggests that, in this tissue, glucose uptake is not dependent upon the presence of insulin. Salivary gland slices produced equal amounts of $^{14}CO_2$ when incubated with glucose-1-^{14}C or glucose-6-^{14}C. In contrast, liver slices produced twice as much $^{14}CO_2$ from glucose-1-^{14}C than from glucose-6-^{14}C. This suggests that, unlike liver tissue, glucose oxidation in salivary gland cells occurs via the Embden-Meyerhoff pathway plus TCA cycle with little or no pentose shunt activity. Although IPR had no demonstrable effect when added to *in vitro,* a single *in vivo* administration of the drug causes a 50% decrease in $^{14}CO_2$ production in salivary gland, but not in liver, slices at 10 hr. A return to normal was seen after 25 hr. Salivary glands that had undergone hypertrophy under the influence of 3 daily injections of IPR, showed no changes in salivary gland glucose oxidation, but an increase in liver activity was seen.

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