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THE LAG PERIOD ASSOCIATED WITH THE STIMULATION OF CEREBRAL GLUCOSE UTILIZATION AND CO_2 FORMATION BY MORPHINE *IN VITRO**

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Abstract—The glucose utilization of cerebral homogenates of rats is increased by morphine *in vitro* in the range of 10^{-6} to 10^{-3} M. Since the kinetic study of this effect revealed that morphine enhances glucose utilization only after a lag period of 30 min, attempts to gain some information about the nature of this lag period were undertaken. The lag period is probably observed, since time is required to form and accumulate some stimulating factor(s) in the reaction mixture, and its formation appears to require ATP, NAD^+, cerebral homogenate, and morphine. Morphine *in vitro* also increases $^{14}CO_2$ formation from glucose-6, -1, or -2-^{14}C. The lag period is associated with the effect of morphine on the $^{14}CO_2$ formation from glucose-6 and -1-^{14}C but not with that from glucose-2-^{14}C. Since the requirements for stimulating the $^{14}CO_2$ formation from glucose-6-^{14}C appear to be the same as those for stimulating glucose utilization, the increase in $^{14}CO_2$ probably reflects the increase in glucose utilization. The increase in $^{14}CO_2$ formation from glucose-1 and -2-^{14}C is difficult to assess, since some of the $^{14}CO_2$ from the first and second carbon atoms of glucose may be metabolized by pathways other than the pentose phosphate cycle or glycolysis.

PREVIOUSLY it was shown that the aerobic and anaerobic glucose uptake of cerebral cortical slices taken from morphinized rats was greater than that of slices taken from control rats. Cerebral slices from animals treated with other central nervous system depressants such as pentobarbital and ethanol did not display an enhanced glucose uptake. The glucose uptake of slices also increased when morphine was added *in vitro*. In addition, morphine *in vitro* also increased the glucose utilization of cerebral homogenates. With homogenates, there was a positive correlation between the glucose utilized and the concentration of morphine *in vitro* in the range of 10^{-6} and 10^{-3} M.[1–3] This puzzling situation whereby morphine, a central depressant, apparently increases cerebral glucose utilization, was investigated further by means of cerebral homogenates.

The present investigation shows that $^{14}CO_2$ formation from labeled glucose is also enhanced by morphine *in vitro*. Kinetic studies of the morphine effect reveal that there is a lag period before the effect of morphine is manifested on either glucose utilization or the $^{14}CO_2$ formation from labeled glucose. Attempts were made to gain some information about the nature of this lag period.

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METHODS

Cerebral homogenates. Male Holtzman rats, 50 to 70 days old, were used in all experiments. Cerebral hemispheres of the rats were rapidly removed, weighed, and homogenized in 9 volumes of ice-cold 0·2 M Na_2HPO_4–KH_2PO_4, K_2HPO_4–KH_2PO_4, or Tris–HCl buffer, pH 7·4, containing 0·01 M nicotinamide. Usually cerebral hemispheres from two rats were pooled to make enough homogenate for any one experiment. A small aliquot of the homogenate was always taken for determining the protein content by the method of Lowry *et al.*[4] The protein content of cerebral homogenates did not vary greatly from sample to sample and was about 12 to 13 mg/ml.

Glucose utilization. The usual reaction mixture for measuring glucose utilization consisted of 12 mM glucose, 4 mM $MgCl_2$, 5 mM ATP, 1 mM NAD^+, 20 mM P_i (inorganic phosphate) as K_2HPO_4–KH_2PO_4 buffer, pH 7·4, and 1 ml cerebral homogenate made in Tris–HCl buffer in a total volume of 2·5 ml. The rate of glucose utilization and the effect of morphine on glucose utilization were determined to be maximum when the above concentration of P_i was employed.[3] The mixture, contained in 25-ml Erlenmeyer flasks, was incubated in a Dubnoff metabolic shaker at 30°, with air as the gas phase. Aliquots of 0·5 ml were taken before and after various times of incubation, and Somogyi's precipitations[5] were performed immediately. Glucose was measured enzymically by a modification of the method of Washko and Rice[6] on the supernatant after centrifugation of the precipitated reaction mixture. Glucose utilization was taken as the difference in glucose content of the medium between zero and various times of incubation.

$^{14}CO_2$ *Formation.* The rate of formation of $^{14}CO_2$ was measured by incubating the above reaction mixture at 30° with 0·05 μc of ^{14}C-labeled glucose in special lipless 20-ml beakers with center wells. The specific position of the label on the glucose molecule is designated in the figure or tables. Enough unlabeled glucose was added to make the final concentration of the glucose 12 mM. The center well contained 0·2 ml of 1 M hydroxide of Hyamine 10-X [*p*-(diisobutylcresoxyethoxyethyl)-dimethylbenzylammonium hydroxide] in methanol and a piece of fluted filter paper to trap the $^{14}CO_2$. The beakers were sealed air-tight with No. 270 "Sani-Tab" rubber caps. The beakers were gassed with O_2 through the caps with two 20-gauge needles. Either trichloroacetic acid (5% final concentration) or perchloric acid (3% final concentration) was added to the main compartments of the beakers through the caps by means of a syringe and needle to stop the reactions after various times of incubation. After the addition of the acid, the incubation was continued for 30 min more to assure complete trapping of the $^{14}CO_2$ formed. The Hyamine carbonate solution and the filter paper in the center well were quantitatively transferred into 10 ml of scintillator fluid, and the radioactive disintegration was determined in a Packard Tri-Carb spectrometer. The samples were counted long enough to yield less than 2 per cent error.

The internal standard was a sample of toluene-^{14}C. The scintillator fluid was made in toluene with either 5 g PPO (2,5-diphenyloxazole) per l. and 0·3 g dimethyl-POPOP [1,4-bis-2-(4-methyl 5 phenyloxazolyl) benzene] per l., or 4 g BBOT {2,5-bis-[5′-tert. butylbenzoxazolyl(2′)]-thiophene} per l. as the scintillator solutes. The results were the same when samples were counted in the two scintillator fluids; however BBOT gave higher counting efficiencies than PPO and dimethyl-POPOP.

Statistics. The data were analyzed statistically by the paired Student's *t*-test.

Chemicals and special materials. ATP and NAD^+ were brought from Sigma Chemical Co. PPO, dimethyl-POPOP, toluene-^{14}C, and Hyamine 10-X were procured from Packard Instrument Co. BBOT was a gift from CIBA Ltd. Labeled glucose was obtained either from New England Nuclear Corp. or Nuclear-Chicago. The special beakers with center wells were those described by Tepperman and Tepperman[7] and were constructed at the glass-blowing shop of the School of Physics of this institution. "Sani-Tab" rubber caps were obtained from Davol Rubber Co.

RESULTS

Kinetic study of glucose utilization

The kinetic study of the increase in glucose utilization due to morphine is illustrated in Fig. 1. The rate of glucose utilization in the presence of morphine is the same

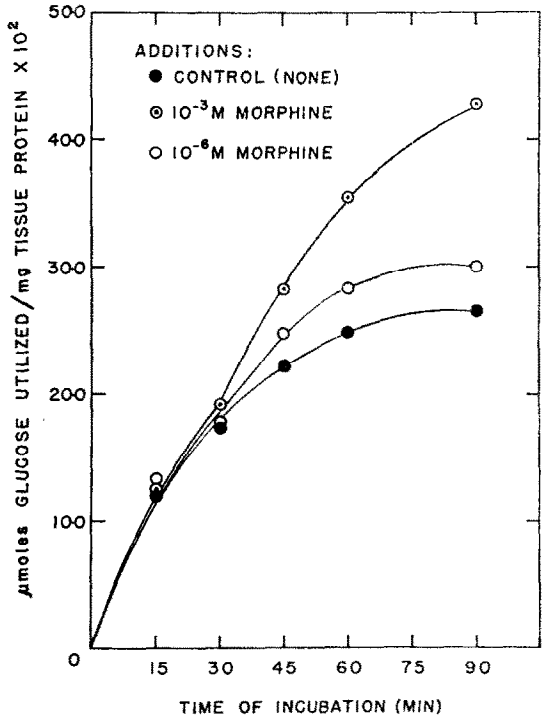

FIG. 1. The kinetic study of the effect of morphine *in vitro* on glucose utilization by cerebral homogenates of rats. The experiments were conducted with homogenates made in 0·2 M Na_2HPO_4–KH_2PO_4 buffer, pH 7·4, containing 0·01 M nicotinamide. The graph represents an average of four experiments.

as that in the absence of morphine during the initial 30 min of incubation. The increase in the rate due to morphine occurs only in the succeeding 30 to 60 min of incubation. Since the rate of glucose utilization in the absence of morphine falls off from linearity after 30 min, morphine, at first glance, appears to just maintain the original rate of glucose utilization longer than 30 min. Thus, when the glucose is measured at any time after 30 min of incubation, an apparent increase in glucose utilization is seen in the presence of morphine.

Effect of various buffers on glucose utilization

When cerebral homogenates are prepared in either K_2HPO_4–KH_2PO_4 or Tris–HCl buffer of equimolar concentrations, the homogenates utilize glucose at a faster rate than those made in Na_2HPO_4–KH_2PO_4 buffer (Table 1). Also, in contrast to homogenates made in Na_2HPO_4–KH_2PO_4 buffer, homogenates made in K_2HPO_4–KH_2PO_4

TABLE 1. EFFECT OF MORPHINE ON GLUCOSE UTILIZATION OF CEREBRAL HOMOGENATES MADE IN VARIOUS BUFFERS

Additions *in vitro*	Time of incubation (min)	μMoles glucose utilized/mg protein × 10^2		
		Na_2HPO_4–KH_2PO_4*	K_2HPO_4–KH_2PO_4*	Tris–HCl*
None (control)	30	21·2	37·7	56·8
	60	32·2	72·1	112·9
10^{-3} M Morphine	30	23·6	39·0	63·9
	60	45·3	100·0	168·5

The values represent an average of four experiments.

* Cerebral homogenates were made in these buffers which were all 0·2 M, pH 7·4, and contained 0·01 M nicotinamide.

or Tris–HCl buffers utilize glucose linearly for at least 60 min. The enhancing effect of morphine on glucose utilization is not only observed but is greater when homogenates are made in K_2HPO_4–KH_2PO_4 or Tris–HCl buffer. Note also that the 30-min lag period is still seen before the effect of morphine becomes apparent.

Effect of morphine on $^{14}CO_2$ formation from labeled glucose

The rate of formation of $^{14}CO_2$ from glucose-^{14}C (uniformly labeled) in the presence of morphine *in vitro* is statistically greater than control (Table 2). A more detailed study of the effect of morphine on the production of $^{14}CO_2$ from differentially labeled

TABLE 2. EFFECT OF MORPHINE ON THE FORMATION OF $^{14}CO_2$ FROM GLUCOSE-^{14}C (U.L.) BY CEREBRAL HOMOGENATES OF RATS

Experiment	Rate of $^{14}CO_2$ formation (dis/min/mg protein/hr)*	
	Control	+ 10^{-3} M Morphine†
1	157·7	206·6
2	116·8	174·5
3	146·9	224·2
4	160·9	203·8
Mean difference	+ 56·7	
Mean % difference	+ 40%	
P value	< 0·01	

Cerebral homogenates were made in 0·2 M Tris buffer, pH 7·4 containing 0·01 M nicotinamide, and 0·05 μc of glucose-^{14}C (uniformly labeled); was included in the reaction mixture. The reaction mixture was incubated for 60 min at 30°.

* Disintegrations per min per mg of homogenate protein per hr. dis/min = counts/min/efficiency where efficiency of the radioactive counting was established with an internal standard.

† Final concentration of morphine added *in vitro*.

glucose is shown in Table 3. Note that the rate of formation of $^{14}CO_2$ from glucose-1-^{14}C is statistically greater than that from glucose-6-^{14}C and that the $^{14}CO_2$ production from glucose-2-^{14}C is even greater than that from glucose-1-^{14}C. The different rates of $^{14}CO_2$ formation from the differentially labeled glucoses occur despite the fact that

TABLE 3. EFFECT OF MORPHINE ON THE RATE OF $^{14}CO_2$ FORMATION FROM DIFFERENTIALLY LABELED GLUCOSES BY CEREBRAL HOMOGENATES

Experiment no.	$^{14}CO_2$ Formation (dis/min/mg protein/hr) from:					
	Glucose-6-^{14}C		Glucose-1-^{14}C		Glucose-2-^{14}C	
	C*	M*	C	M	C	M
1	46·0	70·0	77·4	92·8		
2	42·3	67·5	55·2	89·1		
3	46·7	59·6	74·7	85·3		
4	28·6	56·4	47·0	63·9		
5	51·9	73·5	69·9	81·5	104·0	155·8
6	41·5	53·7	66·6	76·2	123·5	168·1
7	37·1	57·7	47·8	81·7	109·6	158·1
8	30·4	50·7	73·6	97·3	119·2	230·0
9	36·6	54·8			92·2	149·0
10	37·4	72·3			102·1	137·3
Mean difference	+ 21·8		+ 19·5		+ 58·0	
Mean % difference	+ 57%		+ 33%		+ 53%	
P value	< 0·001		< 0·001		< 0·01	

P value for comparison of various groups	Glucose-6-^{14}C vs. glucose-1-^{14}C	Glucose-1-^{14}C vs. glucose-2-^{14}C
	C vs. C $P < 0·001$	C vs. C $P < 0·01$
	M vs. M $P < 0·01$	M vs. M $P < 0·01$
	N = 8	N = 4

Cerebral homogenates were made in 0·2 M Tris buffer, pH 7·4, containing 0·01 M nicotinamide. The amount of labeled glucose added to the medium in each case was 0·05 μc. The reaction mixture was incubated for 60 min at 30°.

* C = Control; M = 10^{-3} M morphine added *in vitro*

the total radioactivity of all three differentially labeled glucoses in the reaction medium as well as the total glucose concentration were the same. Although the rates of formation of $^{14}CO_2$ from the labeled glucoses differ, morphine statistically increases the $^{14}CO_2$ formation from all three types of labeled glucose. There is a greater percentage increase of $^{14}CO_2$ formation from glucose-6-^{14}C than from glucose-1-^{14}C. The percentage increase of $^{14}CO_2$ formation from glucose-2-^{14}C is greater than that from glucose-1-^{14}C and is about the same as that from glucose-6-^{14}C.

Kinetic study of $^{14}CO_2$ formation

The kinetic study of the formation of $^{14}CO_2$ from differentially labeled glucose is presented in Fig. 2. The rate of formation of $^{14}CO_2$ from glucose-6, -1, and -2-^{14}C is linear for 60 min and is stimulated by morphine in each case, as seen previously. There is, however, a lag period of about 30 min before the enhancing effect of morphine on the $^{14}CO_2$ formation from glucose-6 or -1-^{14}C becomes evident. This lag period is similar to the one observed in the earlier studies when the effect of morphine

on glucose utilization was studied. In contrast to the above situation, morphine enhances the $^{14}CO_2$ formation from glucose-2-^{14}C without a lag period.

Preincubation studies

Glucose utilization and the $^{14}CO_2$ formation from labeled glucose were studied further during the initial 30 min of incubation when morphine appeared to have no

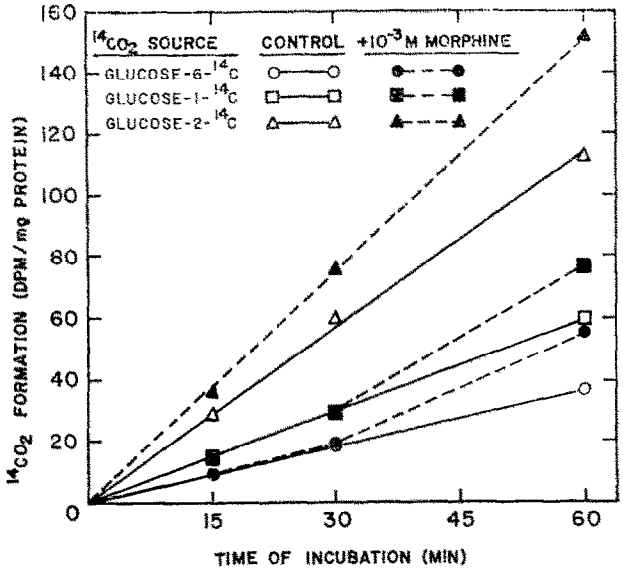

FIG. 2. The kinetic study of the effect of morphine *in vitro* on the formation of $^{14}CO_2$ from differentially labeled glucose by cerebral homogenates of rats. Cerebral homogenates were made in 0·2 M Tris buffer, pH 7·4, containing 0·01 M nicotinamide. The graph represents an average of three experiments.

effect on either utilization or CO_2 production. The reaction mixture described in the Methods section was preincubated for 30 min at 30° in a Dubnoff shaker, during which time various substances of the reaction mixture were omitted. The omitted ingredient was added to the medium after the preincubation period, and glucose utilization or $^{14}CO_2$ formation was measured at the succeeding 30 and 60 min of incubation. If some type of activation took place during the 30-min lag period, one can possibly determine which components of the reaction mixture were necessary for the activation by preincubation studies described above.

When glucose utilization is measured after preincubating the reaction mixture without ATP, NAD^+, or cerebral homogenate, there is still a lag period of 30 min before morphine increases the glucose utilization (Table 4). However, when glucose is left out of the reaction mixture during preincubation, the increase in glucose utilization due to morphine is observed without a lag. The biggest percentage increase in glucose utilization due to morphine is seen when NAD^+ is omitted during preincubation. Although morphine causes only a small percentage increase in glucose utilization when ATP is omitted during preincubation, ATP appears involved with this effect of morphine. The control rate of glucose metabolism is also influenced by the absence

of ATP during preincubation. The rate of glucose utilization is higher after preincubation when ATP is absent rather than present in the reaction mixture during preincubation. The results indicate that something is produced during preincubation that enhances glucose utilization and the formation of this substance seems to require ATP, NAD^+, cerebral homogenate, and morphine.

TABLE 4. PREINCUBATION STUDIES OF GLUCOSE UTILIZATION BY CEREBRAL HOMOGENATES

Ingredient omitted during preincubation*	Time of incubation (min)	μMoles glucose utilized/mg protein × 10^2	
		Control	$+10^{-3}$ M Morphine†
ATP	30	65·5	61·5
	60	105·1	130·0
NAD^+	30	39·1	42·1
	60	78·3	120·2
Homogenate‡	30	49·5	45·0
	60	94·8	124·4
Glucose	30	43·7	60·8
	60	88·7	122·7

The values represent an average of four experiments.

* The ingredients listed in the column were omitted during a preincubation period of 30 min at 30°. The omitted substance was added to the reaction mixture after the preincubation, and glucose utilization was determined at 30 and 60 min of incubation at 30°.

† Morphine at this final concentration was added to the same reaction mixture as the control.

‡ The cerebral homogenate was made in 0·2 M K_2HPO_4–KH_2PO_4 buffer, pH 7·4, containing 0·01 M nicotinamide.

The morphine stimulation of $^{14}CO_2$ formation from glucose-6-^{14}C or glucose-1-^{14}C appears similar to that of glucose utilization (Table 5). There is a lag period before the $^{14}CO_2$ formation is increased by morphine when NAD^+ or cerebral homogenate is omitted from the reaction mixture during preincubation. Morphine increases $^{14}CO_2$ formation without a lag period when glucose is left out of the reaction mixture during preincubation. The requirements for stimulation of $^{14}CO_2$ formation from glucose-6 or -1-^{14}C appear to be the same as those for stimulating glucose utilization except ATP. When ATP is absent during preincubation, the effect of morphine on $^{14}CO_2$ formation disappears.

Since the kinetic study of $^{14}CO_2$ formation from glucose-2-^{14}C indicated that morphine enhances $^{14}CO_2$ formation without a lag period (Fig. 2), one would not expect to observe any kind of stimulating process in the preincubation studies with glucose-2-^{14}C. Morphine indeed does enhance $^{14}CO_2$ formation from glucose-2-^{14}C without delay when $^{14}CO_2$ formation is measured after the preincubating procedure (Table 5).

Another interesting finding is the observation that glucose appears to inhibit its own oxidation. The $^{14}CO_2$ formation from glucose-6, 1-, or -2-^{14}C at 30 min after preincubation without glucose is 10- to 20-fold greater than that after preincubation with glucose (Table 5). The formation of $^{14}CO_2$ from differentially labeled glucoses after preincubation without glucose is about 7-fold greater than even the average

values of $^{14}CO_2$ formation from non-preincubated reaction mixtures observed in Table 3. Preliminary studies also show that with increasing concentrations of glucose in the medium during preincubation, there is a graded decrease in $^{14}CO_2$ formation during the succeeding incubation. In addition, the rate of $^{14}CO_2$ formation is no longer linear for 60 min, and the bulk of the oxidation appears to take place during the initial

TABLE 5. PREINCUBATION STUDIES OF THE FORMATION OF $^{14}CO_2$ FROM DIFFERENTIALLY LABELED GLUCOSES BY CEREBRAL HOMOGENATES

Ingredient omitted during preincubation*	Time of incubation (min)	$^{14}CO_2$ Formation (dis/min/mg protein) from:					
		Glucose-6-^{14}C		Glucose-1-^{14}C		Glucose-2-^{14}C	
		C†	M†	C	M	C	M
ATP	30	11·3	11·2	26·2	24·9	23·9	33·0
	60	27·6	27·9	49·5	48·6	46·7	68·7
NAD^+	30	12·8	13·5	19·2	20·9	30·7	39·8
	60	27·5	33·9	36·7	50·3	60·2	78·9
Homogenates‡	30	19·6	19·5	31·1	30·1	56·4	71·8
	60	40·5	52·2	61·8	79·1	91·6	136·8
Glucose§	30	195·1	262·4	350·3	420·3	501·1	767·3
	60	280·6	457·9	494·6	623·6	575·0	1095·2

The values represent an average of three experiments.

* The ingredients listed in the column were omitted during a preincubation period of 30 min at 30°. The omitted substance was added to the reaction mixture after the preincubation, and the formation of $^{14}CO_2$ was measured at 30 and 60 min of incubation at 30°. All additions were made with a syringe and needle through rubber caps covering the beakers.

† C = Control; M = 10^{-3} M morphine added *in vitro*.

‡ The cerebral homogenate was made in 0·2 M Tris buffer, pH 7·4, containing 0·01 M nicotinamide.

§ Both labeled and unlabeled glucose were omitted during the preincubation period.

30 min after preincubation. Although the $^{14}CO_2$ formation from labeled glucose is very high after preincubating the reaction mixture without glucose, morphine *in vitro* still increases this high rate of $^{14}CO_2$ formation.

DISCUSSION

The initial kinetic study (Fig. 1) indicated that the rate of glucose utilization is not linear beyond 30 min. Therefore, it was initially assumed that the enhancing effect of morphine on glucose utilization is observed only because morphine *in vitro* maintains the linear rate of glucose utilization longer than 30 min. Thus, if one measures glucose utilization at 30 and 60 min of incubation, one would observe an apparent lag period of 30 min before the effect of morphine is seen. The cerebral homogenate in this initial study was made in Na_2HPO_4–KH_2PO_4 buffer and, since Na^+ ions have been shown to inhibit cerebral glycolysis,[8–10] the Na^+ ions probably account for the low rate of glucose utilization by cerebral homogenates (Table 1). Utter[10] has also shown that Na^+ ions stimulate cerebral ATPases, and the concentration of ATP may eventually become limiting to account for the nonlinear utilization of glucose by cerebral homogenates over a period of 60 min. When cerebral homogenates are made in either K_2HPO_4–KH_2PO_4 or Tris–HCl buffer and the reaction mixture incubated

in the absence of Na^+ ions, the glucose utilization by these homogenates is not only greater than that by homogenates made in Na_2HPO_4–KH_2PO_4 buffer but is linear for at least 60 min. Since morphine *in vitro* still increases glucose utilization of homogenates incubated in the absence of Na^+ ions (Table 1), the effect of morphine appears to be more than relieving the inhibition of glycolysis by Na^+ ions.

When the lag period is investigated further, one finds that the lag could be eliminated by preincubating the reaction mixture with ATP, NAD^+, cerebral homogenate, and morphine for 30 min before starting the incubation with glucose. Our preliminary studies of measuring the concentrations of the various intermediate metabolites suggest that the P-fructokinase and the glyceraldehyde-3-P dehydrogenase steps are facilitated by morphine during the second 30 min of incubation, i.e. after the lag period. However, morphine does not appear to enhance the activity of either of these enzymes directly (Dodge and Takemori, unpublished observation). Thus the data from the preincubation studies suggest a formation of a substance during the preincubation period which enhances glucose utilization.

The lag period could possibly be explained if time was needed to accumulate various deinhibitors and stimulators such as FDP, P_i, and ADP which have been shown not only to relieve the ATP inhibition of P-fructokinase but to enhance the activity of P-fructokinase.[11, 12] On the other hand, the amount of P_i included in our reaction mixture (20 mM) would probably deinhibit any inhibition that may be imposed on P-fructokinase by ATP. That ATP does not inhibit P-fructokinase to any extent in our assay system is shown by the fact that the glucose utilization is maximal when the concentration of ATP is highest and when the ATP should be imposing the greatest inhibition on P-fructokinase (Table 4).

Since ATP concentration of the medium remains higher in the presence than in the absence of morphine after 30 min of incubation (Dodge and Takemori, unpublished observation), and since the cerebral ATPases do not appear to be affected by morphine,[3] morphine probably influences regeneration of ATP and may in turn influence glucose metabolism by making more ATP available for use. In this regard, increased ATP levels in the brain are also seen *in vivo* after administration of morphine to rats.[13, 14]

It is difficult to explain why $^{14}CO_2$ formation from glucose-1-^{14}C is greater than that from glucose-6-^{14}C in view of the fact that the pentose cycle is kept dormant in brain, apparently owing to the limited amount of $NADP^+$ available.[15, 16] Although the levels of $NADP^+$ in brain appear to be much higher than the previously reported levels when more sensitive methods are employed for detection, the concentration of $NADP^+$ in the brain is still much lower than in other tissues.[17–20] The obvious explanation is that $NADP^+$ is formed during the incubation and thus stimulates $^{14}CO_2$ formation from glucose-1-^{14}C via the pentose phosphase cycle. However, we could not show the presence of either a NAD-kinase activity or the formation of $NADP^+$ in the reaction mixture. Although our assays were performed fluorometrically, the possibility exists that the method used was not sensitive enough to detect the small amount of $NADP^+$ that was formed in the reaction mixture. The formation of catalytic amounts of $NADP^+$ could conceivably account for the increased $^{14}CO_2$ formation from glucose-1-^{14}C.

The greater rate of formation of $^{14}CO_2$ from glucose-2-^{14}C as compared to that from glucose-1-^{14}C is more difficult to explain. It is hard to visualize the second

carbon atom of glucose being oxidized faster than the first carbon atom. One can explain this observation if one assumes that the first and second and perhaps the third carbon atoms of glucose are metabolized differently from the fourth, fifth, and sixth carbon atoms. The second carbon atom may be oxidized by a pathway other than glycolysis or the pentose phosphate cycle. Recently Morton and Moran[21] have observed that $^{14}CO_2$ production from glucose-2-^{14}C is also higher than that from glucose-1-^{14}C in polymorphonuclear leukocytes and could not explain this observation in terms of the pentose phosphate cycle. The authors also theorize a different metabolic fate for the top three carbon atoms of glucose as compared to the bottom three carbon atoms. Morphine *in vitro* increases the $^{14}CO_2$ formation regardless of the location of the radioactive label on the glucose molecule. However, $^{14}CO_2$ formation is increased by morphine without a lag period only from glucose-2-^{14}C (Fig. 2). This observation also points to the unique oxidation of the second carbon atom of glucose in cerebral tissue.

Since there is a lag period before morphine influences $^{14}CO_2$ formation from glucose-6-^{14}C and the requirements for stimulating $^{14}CO_2$ formation appear the same as those for stimulating glucose utilization, the enhancing effect of morphine *in vitro* on $^{14}CO_2$ formation from glucose-6-^{14}C may be a reflection of the effect of morphine on glucose utilization. The fact that the effect of morphine on $^{14}CO_2$ formation disappears, and that on glucose utilization is relatively small under conditions when ATP was omitted during the preincubation period, may mean that the effect of morphine is seen more readily when the concentration of ATP is low.

The lag seen before morphine increase $^{14}CO_2$ formation from glucose-1-^{14}C may be complicated since, as discussed above, there is a possibility that the first and second carbon atoms of glucose may be metabolized by a pathway other than glycolysis or the pentose phosphate cycle. One must establish the metabolic pathway of the carbon atoms of the top half of glucose in cerebral tissue before one can assess the effect of morphine on CO_2 formation.

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