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EFFECTS OF MORPHINE, NALORPHINE AND PENTOBARBITAL ALONE AND IN COMBINATION ON CEREBRAL GLYCOLYTIC SUBSTRATES AND COFACTORS OF RATS *IN VIVO**

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Abstract—The effects of the administration of morphine, nalorphine and pentobarbital alone and in combination on glycolytic substrate and cofactor levels in rat brain were examined. Pentobarbital increased the concentrations of glucose and glucose 6-phosphate and produced a fall in pyruvate and lactate concentrations. Morphine also increased glucose and glucose 6-phosphate concentrations, but had no effect on either pyruvate or lactate concentrations. Both pentobarbital and morphine produced increases in brain ATP and creatine-phosphate concentrations, but only morphine reduced the concentration of AMP. Nalorphine had no effect on the levels of glycolytic intermediates when given alone, but reversed the changes brought about by morphine. Nalorphine did not reverse the changes produced by pentobarbital. The glycolytic substrate and cofactor levels in brains of chronically morphinized rats did not differ from those in animals that received saline solution over the same period. A challenge dose of 15 mg/kg of morphine administered to the chronically morphinized rats revealed that a large degree of tolerance to the effects of morphine on glycolytic intermediates and cofactors can be demonstrated. The process of withdrawal produced by injections of nalorphine in the chronically morphinized rats did not appear to be related to changes in brain concentrations of glycolytic intermediates.

THE EFFECT of morphine administration on the levels of glycolytic intermediates and energy-rich compounds in the brain of rats has been studied by several investigators.[1,2] Abood *et al.*[1] reported that brains of rats that were given 50 mg/kg of morphine sulfate 5 hr before sacrifice contained more glycogen, fructose diphosphate, pyruvic acid, lactic acid, ATP and ADP and less creatine-phosphate (creatine-P) and glucose 6-phosphate (glucose-6-P) than those of control rats. In contrast, Estler and Ammon[2] showed that, after 45 mg/kg of morphine hydrochloride in rats, there was a small but significant increase in the ATP concentration of brain, while no significant change from control in the concentrations of glycogen, lactate and creatine-P was observed.

The question arises as to whether the changes in glycolytic intermediates seen by the above investigators are related to a possible effect of morphine on special control points of glycolysis, namely the hexokinase, phosphofructokinase (P-fructokinase)

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and glyceraldehyde-3-P dehydrogenase steps.[3–7] We have also shown previously that morphine has a stimulatory effect on rat cerebral glucose metabolism *in vitro*, which involves the glyceraldehyde-3-P dehydrogenase and possibly the P-fructokinase steps.[8]

In the present study, we have compared the effects of pentobarbital, morphine and nalorphine administration on cerebral concentrations of glycolytic substrates and cofactors by using more pharmacologic doses for rats than those employed by previous investigators. In addition, the effects of chronic morphine treatment and precipitation of abstinence by nalorphine on these cerebral substances have been examined.

METHODS

Treatment of animals. Male Holtzman rats, 120–160 g were used in all experiments. Use of larger rats was avoided due to the difficulty of freezing the brain very rapidly. Five groups of animals were acutely treated in the following manner. Animals in group I received 15 mg/kg of morphine sulfate subcutaneously 1 hr prior to sacrifice; those in group II received 10 mg/kg of nalorphine HCl intraperitoneally 30 min prior to sacrifice; those in group III received 15 mg/kg of morphine sulfate subcutaneously 1 hr prior to sacrifice followed by 10 mg/kg of nalorphine HCl intraperitoneally 30 min prior to sacrifice; those in group IV received 35 mg/kg of sodium pentobarbital intraperitoneally 30 min prior to sacrifice; and those in group V received 35 mg/kg of sodium pentobarbital plus 10 mg/kg of nalorphine HCl intraperitoneally 30 min prior to sacrifice. The control group consisted of animals injected with saline solution at the highest volume given in the treated groups.

The chronically morphinized rats received an initial dose of 10 mg/kg twice daily (8 a.m. and 5 p.m.), which was increased in 5 mg/kg increments daily to 30 mg/kg on the fifth day. The dose was then increased in 10 mg/kg increments daily for 7 days to a dose of 100 mg/kg twice daily for 1 day. The control group was chronically treated with saline solution. The chronically treated animals were sacrificed about 16–18 hr after the last dose. The chronically morphinized animals were divided into three groups of which group I received saline solution in the proper volume and time period prior to sacrifice, group II received 15 mg/kg of morphine sulfate subcutaneously 1 hr prior to sacrifice, and group III received 10 mg/kg of nalorphine HCl intraperitoneally 30 min prior to sacrifice.

Preparation of tissue extracts. The procedure followed was essentially that of Lowry *et al.*[5] The rats were decapitated into 2-methyl butane (isopentane), instead of Freon 12, cooled to its freezing point (−150°) in liquid nitrogen. After vigorous stirring for 1 min, the frozen head was removed, placed in aluminum foil and kept on dry ice or at −80° until dissection. The neutralized tissue extracts were prepared exactly as described by Lowry *et al.*[5] in a cryostat maintained at −35°. A portion of the extracts was used immediately for pyruvate determination and the rest were stored at −80°, except for brief intervals when they were thawed to remove aliquants for analysis of other substrates and cofactors.

Analysis of tissue extracts. The analysis of the tissue extracts for the substrates and cofactors of glycolysis was patterned after that previously described by Lowry *et al.*,[5] with the exception that NAD replaced acetyl pyridine NAD in the assay for lactate. Glucose, ATP, creatine-P, lactate, ADP and AMP were analyzed on the Turner model 111 fluorometer as described previously.[8] The volume of tissue extract employed for

each of the assays was: glucose, 100 μl; ATP, 30 μl; lactate, 40 μl; ADP and AMP, 100 μl; and creatine-P, 30 μl.

Fructose diphosphate, 3-P-glyceric acid, pyruvate, glucose-6-P and fructose-6-P were present in concentrations too low for accurate analysis using the Turner fluorometer, so these compounds were assayed on the Farrand model A-3 fluorometer equipped with the Foci microammeter. The volume of tissue extracts employed for each of these analyses was: fructose diphosphate, 25 μl; and 3 P-glyceric acid, pyruvate, glucose-6-P and fructose-6-P, 100 μl. Machine instability was compensated for by the use of a quinine standard. Glucose-6-P and fructose-6-P were determined on the same sample of tissue extract by the addition of phosphohexose isomerase (10 μg/ml) after the change in fluorescence associated with glucose-6-P had been determined.

Substrates and enzymes. All of the substrates and enzymes used in these studies were obtained from either Boehringer Mannheim Corp. or Sigma Chemical Company.

Statistics. All data were statistically analyzed by the Student *t*-test.

RESULTS AND DISCUSSION

The outward signs of drug treatment were observed in each case. Morphine at 15 mg/kg injected acutely produced the usual depressant effect on activity. Nalorphine at 10 mg/kg produced no outward change in the animals when administered alone, but completely reversed the depressant effects of 15 mg/kg of morphine. Pentobarbital at 35 mg/kg produced sleep in all of the animals treated and nalorphine did not reverse this effect. The data were not quantitated, but it appeared that nalorphine accelerated the onset of sleep in the pentobarbital-treated animals.

Pentobarbital at a dose of 35 mg/kg increased the cerebral concentration of glucose, glucose-6-P and fructose-6-P, and decreased the pyruvate and lactate concentrations (Table 1). This apparent inhibition of cerebral glycolytic flux was accompanied by an increase in ATP and creatine-P levels (Table 2). These results are similar to those of Lowry *et al.*[5,6] who concluded that phenobarbital decreased cerebral glucose utilization in mice by inhibiting the phosphofructokinase step of glycolysis.

Morphine at a dose of 15 mg/kg also increased the cerebral concentrations of glucose, glucose-6-P and fructose-6-P, but had no effect on either pyruvate or lactate concentrations (Table 1). Concentrations of ATP and creatine-P were increased and the AMP concentration was significantly lowered (Table 2). That the morphinized animals did not show a fall in lactate and pyruvate levels would suggest that morphine, unlike pentobarbital, does not slow glucose metabolism. According to the data of Abood *et al.*,[1] morphine appears to stimulate the phosphofructokinase step, since they observed a fall in glucose-6-P and a rise in fructose diphosphate and lactate concentrations in brains of morphinized rats. It should be noted, however, that these authors reported the average values for only two animals and employed a rather large dose of morphine (50 mg/kg). Additionally, the tissues were not frozen quickly by immersion in liquid nitrogen, but were frozen between two pieces of dry ice. Lowry *et al.*[5] have since shown that huge changes in glycolytic substrate and cofactor concentrations occur during the very brief period of ischemia prior to freezing. In this regard, Estler and Ammon[2] also showed that morphine in rats did not alter the brain concentration of lactate. They also observed an increase in ATP levels, but not in creatine-P levels.

The marked increase in brain glucose concentration could not be attributed to an increase in blood glucose concentration, since neither morphine nor pentobarbital

TABLE 1. EFFECTS OF MORPHINE, NALORPHINE AND PENTOBARBITAL ALONE AND IN COMBINATION ON CEREBRAL GLYCOLYTIC SUBSTRATE LEVELS*

Substance	Rats	Treatment	Cerebral concn (μmoles/kg wet wt.)
Glucose	(10)	C	365 ± 41
	(5)	M	1239 ± 94†
	(5)	N	453 ± 22
	(5)	M + N	662 ± 112
	(10)	P	1090 ± 142†
	(5)	P + N	1280 ± 96†
Glucose-6-P	(10)	C	21·1 ± 5·3
	(5)	M	46·9 ± 4·2†
	(5)	N	35·8 ± 5·3†
	(5)	M + N	27·8 ± 1·9
	(10)	P	34·3 ± 2·7†
	(5)	P + N	36·2 ± 3·2†
Fructose-6-P	(5)	C	11·6 ± 1·0
	(5)	M	24·7 ± 3·0†
	(5)	N	14·6 ± 3·0
	(5)	M + N	9·6 ± 1·5
	(5)	P	20·2 ± 2·5†
Fructose diphosphate	(5)	C	66·0 ± 8·3
	(5)	M	60·1 ± 7·6
	(5)	N	65·7 ± 10·0
	(5)	M + N	67·1 ± 9·3
	(5)	P	67·7 ± 11·3
3-P-glycerate	(5)	C	30·3 ± 3·8
	(5)	M	35·2 ± 3·9
	(5)	N	22·7 ± 9·8
	(5)	M + N	35·7 ± 7·2
	(5)	P	33·9 ± 2·7
Pyruvate	(5)	C	34·0 ± 2·6
	(5)	M	33·4 ± 3·8
	(5)	N	34·8 ± 5·0
	(5)	M + N	33·0 ± 3·0
	(5)	P	20·8 ± 2·6†
Lactate	(5)	C	3072 ± 145
	(5)	M	3307 ± 352
	(5)	N	3324 ± 485
	(5)	M ± N	3940 ± 471
	(5)	P	1794 ± 259†

* The numbers of animals in each group are indicated in parentheses. The symbols used in the table are as follows: C = control (saline-treated rats); M = morphine-treated rats; N = nalorphine-treated rats; M + N = rats treated with morphine and nalorphine; P = pentobarbital-treated rats; P + N = rats treated with pentobarbital and nalorphine. For further details, refer to the Methods section.

† Values are significantly different from control values ($P < 0·05$).

TABLE 2. EFFECTS OF MORPHINE, NALORPHINE AND PENTOBARBITAL ALONE AND IN COMBINATION ON CEREBRAL GLYCOLYTIC COFACTOR LEVELS*

Substance	Rats	Treatment	Cerebral concn (μmoles/kg wet wt.)
Creatine phosphate	(10)	C	3815 ± 362
	(5)	M	5006 ± 389†
	(5)	N	4743 ± 289
	(5)	M + N	5220 ± 314†
	(10)	P	5851 ± 232†
	(5)	P + N	5682 ± 283†
ATP	(10)	C	1081 ± 205
	(5)	M	1642 ± 92†
	(5)	N	1184 ± 163
	(5)	M + N	1153 ± 153
	(10)	P	1886 ± 75†
	(5)	P + N	1910 ± 83†
ADP	(5)	C	845 ± 68
	(5)	M	901 ± 52
	(5)	N	779 ± 64
	(5)	M + N	862 ± 48
	(5)	P	767 ± 28
AMP	(5)	C	504 ± 31
	(5)	M	394 ± 34†
	(5)	N	418 ± 41
	(5)	M + N	424 ± 29
	(5)	P	433 ± 27

* See legend under Table 1.
† Values are significantly different from control values ($P < 0.05$).

at the doses employed in this study altered blood glucose levels. The control level of blood glucose was 145 ± 13 mg/100 ml, while the blood levels for animals treated with morphine and pentobarbital were 163 ± 18 and 167 ± 15 mg/100 ml respectively. Thus, the possibility that morphine may increase glucose transport into the brain must be entertained. With regard to barbiturates and other anesthetics, Mayman *et al.*[9] have calculated that the large increase in brain glucose concentration that was observed after the anesthetic agents could not be due solely to the decreased rate of glucose utilization. They suggested the possibility that anesthetics increased the glucose transport into the brain. The possible facilitation of glucose transport by morphine occurs apparently without a slowing of glucose consumption.

Further evidence that the effect of morphine on glycolysis differs from that of pentobarbital is the fact that nalorphine reversed all the changes on glycolytic intermediates produced by morphine except the increase in creatine-P. Nalorphine had no influence on the changes produced by pentobarbital and also had no effect of its own on the levels of glycolytic intermediates (Tables 1 and 2). It is difficult to explain why the change in creatine-P was not reversed by nalorphine; however, Estler and Heim[10] were able to show in mice that levallorphan can reverse the changes in creatine-P as well as in ATP produced by morphine.

It was observed earlier that morphine influenced glucose metabolism *in vitro*, at least in part, by stimulating the glyceraldehyde-3-P dehydrogenase step.[8] The low AMP concentration in the presence of morphine was thought to increase the glyceraldehyde-3-P dehydrogenase step indirectly, since AMP inhibited the action of this enzyme. Cerebral AMP concentration was significantly decreased after morphine administration (Table 2), but 3-P-glycerate concentration was not altered (Table 1). These results indicate that, although similar alterations in adenine nucleotides were observed *in vitro* and *in vivo*, compensatory mechanisms are such that morphine does not influence the glyceraldehyde-3-P dehydrogenase step *in vivo* and the stimulatory effect can only be observed under the specific conditions of the studies *in vitro*. The

TABLE 3. EFFECTS OF CHRONIC MORPHINIZATION AND ACUTE WITHDRAWAL ON CEREBRAL GLYCOLYTIC SUBSTRATE LEVELS*

Substance	Rats	Treatment	Cerebral concn (μmoles/kg wet wt.)
Glucose	(3)	CS	369 ± 28
	(5)	MS	302 ± 18
	(4)	MM	566 ± 34†
	(4)	MN	646 ± 40†
Glucose-6-P	(3)	CS	26·0 ± 4·1
	(5)	MS	32·0 ± 2·7
	(4)	MM	48·0 ± 8·4†
	(4)	MN	38·4 ± 7·2
Fructose diphosphate	(3)	CS	96·3 ± 7·2
	(5)	MS	103·2 ± 11·3
	(4)	MM	107·0 ± 9·0
	(4)	MN	94·2 ± 7·0
3-P glycerate	(3)	CS	24·6 ± 3·3
	(5)	MS	29·9 ± 7·8
	(4)	MM	33·3 ± 5·1
	(4)	MN	33·4 ± 4·5
Pyruvate	(3)	CS	46·1 ± 5·7
	(5)	MS	45·8 ± 2·7
	(4)	MM	46·8 ± 3·9
	(4)	MN	40·7 ± 7·1
Lactate	(3)	CS	2535 ± 212
	(5)	MS	3274 ± 143
	(4)	MM	2567 ± 155
	(4)	MN	3233 ± 290

* The numbers of animals in each group are indicated in parentheses. The symbols used in the table are as follows: CS = rats chronically treated with saline solution; MS = chronically morphinized rats which were injected with saline prior to sacrifice; MM = chronically morphinized rats which were injected with morphine prior to sacrifice; MN = chronically morphinized rats which were injected with nalorphine prior to sacrifice. For further details, refer to the Methods section.

† Values are significantly different from control (MS) values ($P < 0.05$).

effect seen with morphine could actually be a complicated net effect involving the slowing of glycolysis at the phosphofructokinase step, much like the effect observed with pentobarbital, and an apparent stimulation of the glyceraldehyde-3-P step as seen previously *in vitro*. This may be the reason why morphine administration failed to decrease pyruvate and lactate levels like with pentobarbital administration.

The effects of chronic morphinization on cerebral glycolytic intermediates were also studied. The chronically morphinized animals weighed less than those chronically treated with saline solution due to a reduced food intake during the injection schedule. Other than this fact, there did not appear to be any difference in the gross appearance of the animals. Morphine at a dosage of 15 mg/kg did not affect the chronically morphinized animals, which indicated that they were tolerant to the depressant effect usually seen at this dose. Nalorphine precipitated signs of withdrawal in the chronically morphinized animals which were characterized by extreme agitation and profuse diarrhea.

When the brains of chronically morphinized rats were analyzed for glycolytic intermediates, there was no significant effect on the concentrations of any of the substances previously studied in Tables 1 and 2 (Tables 3 and 4). However, an acute dose of morphine administered to the chronically treated animals still produced a significant increase in the brain glucose and glucose-6-P concentrations (Table 3). The increase in glucose level was only 54 per cent compared to an over 3-fold increase in acutely morphinized animals (Table 1).

These increases in brain glucose and glucose-6-P concentration caused by an acute dose of morphine may indicate that the animals were in the early stages of abstinence.

TABLE 4. EFFECTS OF CHRONIC MORPHINIZATION AND ACUTE WITHDRAWAL ON CEREBRAL GLYCOLYTIC COFACTOR LEVELS*

Substance	Rats	Treatment	Cerebral concn (μmoles/kg wet wt.)
ATP	(3)	CS	1107 ± 102
	(5)	MS	1263 ± 111
	(4)	MM	1536 ± 272
	(4)	MN	1308 ± 277
ADP	(3)	CS	914 ± 38
	(5)	MS	996 ± 28
	(4)	MM	1010 ± 22
	(4)	MN	942 ± 75
AMP	(3)	CS	810 ± 44
	(5)	MS	711 ± 32
	(4)	MM	726 ± 127
	(4)	MN	695 ± 71
Creatine-P	(3)	CS	3557 ± 327
	(5)	MS	4023 ± 429
	(4)	MM	4858 ± 723
	(4)	MN	5069 ± 666

* See legend under Table 3.
† Values are significantly different from control (MS) values ($P < 0.05$).

As was mentioned previously, there is a 16-hr lag period between the last dose of the chronic morphinization schedule and the acute dose of morphine.

Precipitation of abstinence signs in the chronically morphinized rats by the injection of nalorphine did not produce any changes in the glycolytic substrate and cofactor levels in the brain (Tables 3 and 4) except in the glucose concentration, which was elevated (Table 3). Thus, the process of withdrawal does not appear to be related to changes in concentrations of these substances.

In conclusion, morphine has a definite effect on cerebral glucose metabolism and possibly on central glucose transport as well. The effect of morphine on the glyceraldehyde-3-P dehydrogenase step which was observed previously *in vitro* is not apparent *in vivo*. However, the effect of morphine may actually be a complicated net effect at two control points in glycolysis, namely the phosphofructokinase and glyceraldehyde-3-P steps. A large accumulation of glucose in the brain results from the administration of morphine or pentobarbital, and this may be due mainly to a nonspecific increase in central glucose transport caused by all central nervous system depressants. The effects of morphine on glycolytic intermediates can be reversed by the administration of nalorphine, but those of pentobarbital cannot. The formation of tolerance to the effects of morphine can be demonstrated.

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