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Mechanism of Inhibition of Protein Synthesis by Morphine in Rat Brain and Liver

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SUMMARY

Acute administration of morphine (30 mg/kg) to rats produced an 80% decrease in the rate of secretory and nonsecretory protein synthesis in liver after 30, 60, 90, and 120 min, but did not affect the rate in brain. The inhibition of protein synthesis was attributable to a decreased rate of polypeptide elongation. At a lower dose of morphine (10 mg/kg) the rate of protein synthesis in liver was almost normal. The mechanisms by which protein synthesis could be inhibited were investigated. Morphine, at concentrations ranging from 10^{-5} to 10^{-3} M, did not affect the rate of protein synthesis in either the rabbit reticulocyte lysate or wheat germ embryo translation system, suggesting that the response in the intact animal is due to an effect of the drug on some physiological process. This possibility was investigated by examining acute effects (30 min) of treatments that mimic or prevent morphine action on physiological processes. Acetazolamide (100 mg/kg) produced a degree of acidosis and hypercapnia similar to that obtained with morphine (30 mg/kg), but did not affect the rate of protein synthesis in liver or brain. On the other hand, exposure of rats to an atmosphere of 10% O_2/90% N_2 produced a degree of hypoxia similar to that obtained with morphine (30 mg/kg) and a 50% decrease in the rate of protein synthesis in liver, but did not affect the rate in brain. Moreover, treatments that blocked morphine-induced respiratory depression (i.e., concomitant administration of the opiate antagonist naloxone) or that elevated arterial P_{O_2} in morphine-treated rats (i.e., concomitant administration of 95% O_2/5% CO_2) prevented inhibition of hepatic protein synthesis. Thus, we conclude that morphine inhibits hepatic protein synthesis by depressing respiration and producing systemic hypoxia. Because energy production normally limits the rate of protein synthesis in hypoxic tissues, the effects of the previous treatments on ATP concentration were investigated. All treatments that produced inhibition of hepatic protein synthesis (morphine, 30 mg/kg, and 10% O_2/90% N_2) caused a 50% decrease in the concentration of ATP in liver, but did not affect the concentration in brain, whereas all treatments that prevented inhibition (naloxone plus morphine and morphine plus 95% O_2/5% CO_2) did not affect the concentration of ATP in either tissue. Furthermore, morphine treatment in a 37° environment produced a 60% decrease in the rate of protein synthesis and a 20% decrease in the concentration of ATP in brain, and greater decreases in both parameters in liver than obtained in a 24° environment. Thus, we conclude that morphine-induced hypoxia inhibits hepatic protein synthesis by interfering with the regeneration of ATP by aerobic pathways, but does not normally affect cerebral protein synthesis because it induces a compensatory increase in cerebral blood flow that maintains the tissue in energy balance.

INTRODUCTION

Several studies have shown that opiates inhibit RNA and protein synthesis in mammalian cells in culture (1–3). This has raised the question of whether inhibition of cerebral protein synthesis is required for the expression of the acute effects of morphine. Although there is evidence that acute morphine administration reduces the incorporation of labeled amino acids into cerebral (4–10) and hepatic (5, 8, 11) proteins, it is not clear whether these responses are a result of an actual decrease in the rate of protein synthesis or are due to other factors, i.e., differences in amino acid uptake, compartmentation, or reutilization. Furthermore, attempts to understand the mechanisms underlying these responses have been unsuccessful, although one possibility, that of morphine-

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induced hypothermia, has been eliminated (5). The answers to these questions may be helpful in understanding the significance of the acute effects of morphine.

In the present study we have examined the rate of protein synthesis *in vivo* in rat brain and liver after morphine and other treatments that mimic or prevent morphine action on physiological processes. Rates of protein synthesis on free and membrane-bound polysomes were determined 6 min after i.v. administration of a dose (500 μmole/kg) of low specific activity leucine, a treatment which expands the intracellular pool of leucine and maintains its specific activity constant during the period of measurement (12). This approach not only eliminated the need to know the specific activity of leucine in the endogenous amino acid pool, but also minimized possible problems associated with differences in leucine uptake, compartmentation, metabolism, and reutilization. Moreover, the pool-expansion approach provided a means of determining actual rates of protein synthesis and sites of inhibition of protein synthesis.

MATERIALS AND METHODS

Animals and treatments. Male Sprague-Dawley rats (Biolab, Minneapolis, Minn.) weighing 130–150 g were housed in stainless steel cages and maintained in 12-hr light and dark cycles with free access to food and water for at least 2 days prior to use. Drugs were administered by s.c. injection along the lower dorsal midline in a volume of 2 ml of 0.9% NaCl/kg body weight except as noted below. Doses of morphine given as the sulfate salt (Merck, Sharp & Dohme, Rahway, N. J.) refer to the free base; doses of other drugs refer to their respective salts. Drug dosages were as follows: morphine, 10 and 30 mg/kg; naloxone (a gift from Endo Laboratories, Garden City, N. Y.), 6 mg/kg given in two doses of 3 mg/kg 20 min apart; acetazolamide (Lederle Laboratories, Pearl River, N. Y.), 100 mg/kg given in a volume of 1 ml/kg i.p. In studies with morphine and naloxone, naloxone (3 mg/kg) was given 5 min before and 15 min after morphine; 0.9% NaCl was given in place of morphine when the effects of naloxone were investigated.

Measurement of rate of protein synthesis in vivo. Thirty minutes after initiating treatments (35 min in experiments with naloxone), each rat received an i.v. injection (jugular vein) of low specific activity [4,5-^{3}H] leucine (1 mCi/kg of 2 Ci/mole in 3.3 ml of 0.9% NaCl/kg) under ether anesthesia, using a circulating air-ether or experimental gas-ether mixture. Six minutes after receiving isotope, the rats were perfused with ice-cold 250 mM sucrose/1 mM $MgCl_2$ for 45 sec via the portal vein under ether anesthesia to terminate protein synthesis. Brain and liver were excised and maintained at 0° in perfusion medium; subsequent steps were performed at 0–4°.

Homogenates of brain (10%, w/v) and liver (20%, w/v) were prepared and centrifuged to separate free polysomes (nonsecretory protein-synthesizing compartment) from membrane-bound polysomes (secretory/membrane protein-synthesizing compartment) as described previously by Ramsey and Steele for brain (13) and liver (14). Aliquots of the two compartments (2 ml) were centrifuged at 1° for 75 min at 226,000 × g_{max} to remove all ribosomes, and aliquots of that supernatant were used to determine released protein radioactivity by the method of Mans and Novelli (15) followed by digestion of the protein with 0.5 ml of NCS (Amersham/Searle, Arlington Heights, Ill.) at 37° for 18 hr, acidification, and counting in toluene fluor (16). Another set of aliquots (3 ml) was layered over 4 ml of 1.38 M sucrose containing high-salt medium and centrifuged at 1° for 4 hr at 226,000 × g_{max} to isolate and purify the polysomes (13, 14). The pellets were dissolved in 0.5% (w/v) sodium dodecyl sulfate, and aliquots of that solution were used to determine both nascent chain radioactivity using Aquasol fluor (Amersham/Searle) and ribosomal RNA recovery from the absorbance at 260 nm, assuming that 20 A_{260} units = 1 mg of RNA. Prior to tissue fractionation, aliquots (100 μl) of homogenate were placed on filter paper discs, digested with 1 ml of NCS, acidified, and counted as above to determine intracellular radioactivity.

Display and analysis of polysomes. Equal portions of each polysome compartment and its corresponding ribosome-free supernatant (prepared as described above) were layered over identical 36-ml 20–47% (w/w) linear sucrose gradients containing high-salt medium and centrifuged in opposite buckets of the same SW 27 rotor (Beckman) at 1° for 4.5 hr at 131,000 × g_{max} (13). After centrifugation, each set of gradients was monitored simultaneously (sample minus blank) at 254 nm with a dual-beam absorbance analyzer (Model UA-5, Instrumentation Specialties Co., Lincoln, Nebr.) to obtain a difference profile (17). The polysome content, i.e., the proportion of polysomes relative to the total complement of ribosomal components, was determined by planimetry of the difference profile (9862A Calculator Plotter; Hewlett Packard, Palo Alto, Calif.) and expressed as a percentage of the total area under the profile.

Cell-free protein-synthesizing systems. The preparation of the rabbit reticulocyte lysate and wheat germ embryo translation systems as well as the conditions for incubation and measurement of incorporation were essentially as described by Pelham and Jackson (18) and Marcu and Dudock (19), respectively. Incubations were performed with [^{3}H]leucine and poly(A^+) mRNA isolated from rat liver free polysomal RNA by chromatography on oligo(dT) cellulose.

Assay of tissue adenylate nucleotides. Adenylate nucleotides were determined in separate experiments using treatment procedures identical with those described above except that nonradioactive leucine was used and perfusion was omitted. Liver (one lobe) was frozen *in situ* with a freeze-clamp (20) and stored in liquid nitrogen; the rat was then decapitated and the brain was excised and dropped into liquid nitrogen; all sampling was completed within 20 sec. The frozen samples were shattered with a hammer on a precooled (liquid nitrogen) aluminum plate and extracted with 0.3 N $HClO_4$ in a blender (Sorvall Omni-Mixer) at maximal speed for 5 min at 4°. The extracts were centrifuged at 1° for 10 min at 4500 × g_{max}, and aliquots of that supernatant were neutralized with 2.5 N KOH at 0° and centrifuged to remove $KClO_4$. Adenylate nucleotides were assayed by the luciferase enzyme system described by Kimmich *et al.* (21).

Blood gas analysis. Blood (0.5–1 ml) was sampled

from the lower abdominal aorta with a heparinized syringe just prior to perfusion and analyzed for pH, P_{CO_2} and P_{O_2} with a blood gas analyzer (Model 213, Instrumentation Laboratory, Lexington, Mass.).

Statistical analyses. Statistical comparisons between two means were made using a two-tailed Student's *t*-test, $p < 0.05$. Comparisons between three or more means were made using a one-way analysis of variance and the Student-Newman-Keul's test, $p < 0.05$.

RESULTS

Effects of Morphine on Rate of Protein Synthesis in Vivo

To ascertain whether morphine affects the rate of protein synthesis, each rat received an injection of a pool expansion dose of [^{3}H]leucine at time intervals ranging from 30 to 120 min after treatment with morphine in an ambient temperature of 24°, and the incorporation into nascent chains and released proteins by free and membrane-bound polysomes and the amount of intracellular radioactivity were determined following a 6-min pulse. For this time period, released protein radioactivity is a measure of the rate of protein synthesis, since nascent chains are uniformly labeled within 3 min and their specific radioactivity remains constant for at least another 6 min, whereas total protein (nascent chains plus released proteins) is labeled at a linear rate throughout the first 9 min (12).

Table 1 shows that morphine, at doses of 10 and 30 mg/kg, did not affect the rate of protein synthesis in brain at 30 min after administration (lines 2 and 3 compared with line 1); similar results were obtained after 60, 90, and 120 min (data not presented), ruling out delayed effects. Table 2 shows, in contrast, that a 10 mg/kg dose of morphine did not significantly affect the rate of protein synthesis in liver, whereas a 30 mg/kg dose of morphine reduced the rate on both free and membrane-bound polysomes by about 80%, but did not affect the amount of intracellular radioactivity (data not presented). Similar results were obtained after 60, 90, and 120 min (data not presented). Hence, in the experiments *in vivo* which follow, a 30-min treatment period was used in order to minimize problems associated with prolonged inhibition of protein synthesis.

Before proceeding with the analysis of the mechanism by which morphine reduces the rate of protein synthesis in liver, it is useful to know which step in protein synthesis is inhibited. Analysis of polysome size and nascent chain synthesis and completed protein release can be used to determine the site of inhibition. Fig. 1a–d shows representative profiles of the total complement of ribosome species in free and membrane-bound polysome compartments of brain (a and b) and liver (c and d) from rats given morphine (30 mg/kg) or 0.9% NaCl as in the previous experiments. In all cases, the major features of the profiles from treated and control rats are similar, and in numerous experiments both the average size of the polysomes and the content of polysomes (Table 3) have in all cases been similar. Thus, the average size of liver polysomes was maintained in spite of a 5-fold reduction in the rate of protein synthesis. The maintenance of polysome size when the rate of protein synthesis decreased is an indication that elongation or termination rather than initiation was inhibited. To choose between these alternatives, nascent chain synthesis and completed protein release were analyzed. Table 2 shows that morphine (30 mg/kg) reduced nascent chain synthesis and completed protein release to about the same extent, and decreased the ratio of r/n by only 30%. Thus, elongation rather than termination was inhibited, since if the

TABLE 1

Effects of morphine and other treatments on the rate of protein synthesis in free and membrane-bound polysome compartments of rat brain in vivo

Rats were treated as described under Materials and Methods. Thirty minutes after initiating treatments (35 min in experiments with naloxone), the rats were pulse-labeled with [^{3}H]leucine for 6 min. Brains were processed for separation of free and membrane-bound polysome compartments and subsequently for isolation of polysomes and released protein as described under Materials and Methods. The values are the means ± standard error of determinations on three to six animals.

Conditions			Free polysome compartment			Bound polysome compartment		
Environmental temperature	Atmosphere	Drug and dose (mg/kg)	Released protein	Nascent chains	r/n	Released protein	Nascent chains	r/n
			dpm/mg RNA × 10^{-3}			*dpm/mg RNA × 10^{-3}*		
22°	Air	Control	34.7 ± 1.6	10.1 ± 0.8	3.4	40.2 ± 3.1	14.1 ± 1.0	2.9
22	Air	Morphine (10)	36.7 ± 4.3	11.9 ± 0.9	3.9	37.3 ± 2.3	15.1 ± 2.8	2.5
22	Air	Morphine (30)	37.0 ± 3.4	10.1 ± 1.0	3.7	41.2 ± 3.7	13.1 ± 1.0	3.1
22	Air	Acetazolamide (100)	36.7 ± 1.5	10.0 ± 0.7	3.7	40.9 ± 1.5	15.1 ± 0.7	2.7
22	10% O_2/90% N_2	—	33.0 ± 3.5	9.7 ± 0.2	3.4	40.0 ± 2.7	14.7 ± 0.5	2.7
22	Air	Naloxone (6)	31.8 ± 2.0	11.0 ± 1.9	2.9	40.9 ± 2.9	13.2 ± 2.4	3.1
22	Air	Naloxone (6) + morphine (30	32.4 ± 2.3	10.3 ± 0.9	3.2	40.0 ± 3.7	13.3 ± 1.1	3.0
22	95% O_2/5% CO_2	Control	40.4 ± 0.7	10.8 ± 1.1	3.7	45.2 ± 11.0	11.4 ± 1.4	4.0
22	95% O_2/5% CO_2	Morphine (30)	37.6 ± 3.3	11.5 ± 0.7	3.3	38.8 ± 2.5	12.4 ± 1.0	3.1
37	Air	Control	35.9 ± 5.5	14.8 ± 1.7	2.4	45.1 ± 8.2	12.9 ± 0.3	3.5
37	Air	Morphine (30)	13.2 ± 3.8[a]	6.6 ± 2.4[a]	2.0	18.2 ± 4.4[a]	7.4 ± 1.1	2.5

[a] Treatment different from respective control, Student's *t*-test, $p < 0.05$.

TABLE 2

Effects of morphine and other treatments on the rate of protein synthesis in free and membrane-bound polysome compartments of rat liver in vivo

Rats were treated as described in Table 1 and under Materials and Methods. Livers were processed as described under Materials and Methods. The values are the means ± standard error of determinations on three to six animals.

Conditions			Free polysome compartment			Bound polysome compartment		
Environmental temperature	Atmosphere	Drug and dose (mg/kg)	Released protein	Nascent chains	r/n	Released protein	Nascent chains	r/n
			dpm/mg RNA × 10^{-3}			*dpm/mg RNA × 10^{-3}*		
22°	Air	Control	59.8 ± 3.5	8.5 ± 1.0	7.1	57.0 ± 3.7	10.7 ± 1.3	5.3
22	Air	Morphine (10)	50.8 ± 4.5	7.0 ± 1.7	7.3	50.8 ± 7.8	8.8 ± 0.5	5.8
22	Air	Morphine (30)	11.3 ± 2.8[a]	2.2 ± 0.4[a]	5.1	12.7 ± 3.8[a]	3.3 ± 0.8[a]	3.8
22	Air	Acetazolamide (100)	52.1 ± 3.4	8.3 ± 0.3	6.2	47.1 ± 2.8	10.6 ± 0.4	4.4
22	10% O_2/90% N_2	—	28.5 ± 1.8[a]	8.0 ± 0.2	3.5	26.1 ± 1.2[a]	10.6 ± 0.4	2.5
22	Air	Naloxone (6)	51.8 ± 4.5	8.5 ± 0.6	6.1	58.4 ± 0.6	10.9 ± 1.7	5.4
22	Air	Naloxone (6) + morphine (30)	51.3 ± 3.4	8.2 ± 0.3	6.2	54.7 ± 0.4	10.7 ± 0.8	5.1
22	95% O_2/5% CO_2	Control	73.2 ± 5.4	10.9 ± 1.1	6.4	69.0 ± 7.2	14.6 ± 1.6	4.7
22	95% O_2/5% CO_2	Morphine (30)	58.9 ± 10.1	10.1 ± 1.0	5.8	53.8 ± 9.3	13.4 ± 0.5	4.0
37	Air	Control	61.3 ± 2.5	10.6 ± 1.3	5.8	56.8 ± 9.1	12.7 ± 1.0	4.5
37	Air	Morphine (30)	2.7 ± 0.1[b]	0.7 ± 0.1[b]	4.1	2.0 ± 0.2[b]	1.0 ± 0.1[b]	1.9

[a] Treatment different from respective control, one-way analysis of variance $p < 0.05$.
[b] Treatment different from respective control, Student's t-test, $p < 0.05$.

major effect of morphine were to reduce the rate of termination, completed protein release should be much lower than nascent chain synthesis, and the ratio of r/n should fall by about 80% for a 5-fold reduction in the rate of protein synthesis.

Mechanism of Inhibition of Protein Synthesis by Morphine

There are two basic mechanisms by which acute morphine administration could reduce the rate of protein synthesis. It could inhibit the function of the protein synthetic machinery or reduce the concentration of some factor required for protein synthesis. The first possibility can be investigated by examining protein synthesis *in vitro*.

Effect of morphine on protein synthesis in vitro. To ascertain whether morphine affects the activity of the protein synthetic machinery, aliquots of rabbit reticulocyte lysate and wheat germ embryo translation systems were incubated for 30 and 60 min with concentrations of

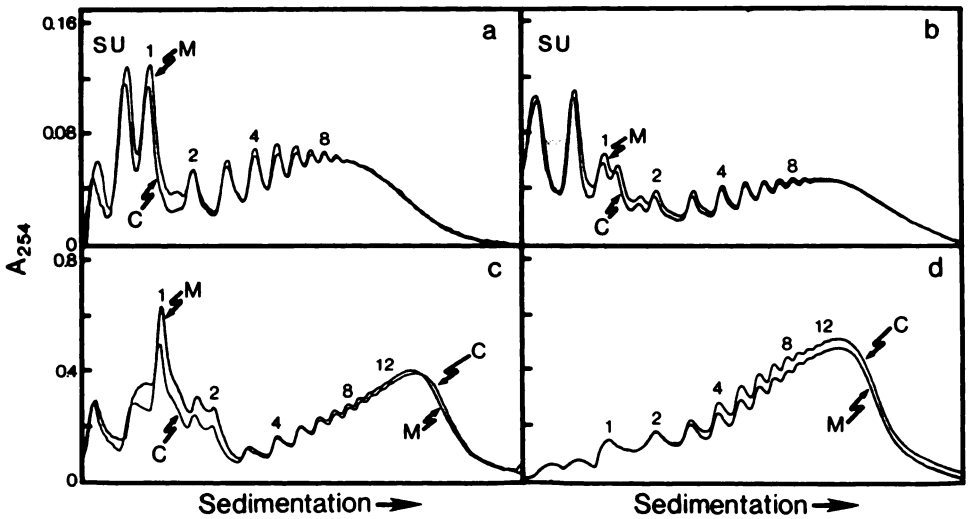

FIG. 1. *Effect of acute morphine administration on the size and amount of free and membrane-bound polysomes in rat brain and liver*

Morphine (30 mg/kg) was administered to one rat, while another rat received an injection of 0.9% NaCl and served as control. Thirty minutes later the brains and livers were excised, and the free and membrane-bound polysome compartments were prepared as described under Materials and Methods. Equal portions of each polysome compartment and its ribosome-free supernatant were displayed on sucrose gradients and monitored simultaneously (sample minus blank) at 254 nm to obtain a difference profile. a and b, Free and membrane-bound polysome compartments from brain; c and d, from liver. *M*, A_{254} profile from morphine-treated rat; *C*, that of control. *SU*, Ribosomal subunits; *1*, monosomes; *2*, *4*, *8*, *12*, di-, tetra-, octa-, and dodecasomes.

TABLE 3

Effects of morphine and other treatments on the polysome content of free and membrane-bound polysome compartments of rat liver and brain

Rats were treated as described in Table 1 and under Materials and Methods. Analyses are described under Materials and Methods. The values are the means ± standard error of determinations on three to six animals.

Conditions			Liver		Brain	
Environmental temperature	Atmosphere	Drug and dose (mg/kg)	Free	Bound	Free	Bound
			%	%	%	%
22°	Air	Control	65.7 ± 1.6	88.4 ± 1.2	72.3 ± 0.8	69.9 ± 1.4
22	Air	Morphine (10)	66.3 ± 1.3	83.3 ± 4.1	69.8 ± 1.7	68.2 ± 3.7
22	Air	Morphine (30)	60.0 ± 3.2	31.9 ± 3.8	69.0 ± 1.4	65.6 ± 2.8
22	Air	Acetazolamide (100)	67.0 ± 3.3	86.7 ± 1.0	69.6 ± 1.3	70.6 ± 2.3
22	10% O_2/90% N_2	—	65.5 ± 2.0	87.6 ± 1.4	68.9 ±0.9	67.0 ± 2.3
22	Air	Naloxone (6)	62.5 ± 1.9	91.1 ± 3.1	69.9 ± 2.9	66.8 ± 2.9
22	Air	Naloxone (6) + morphine (30)	61.1 ± 3.5	85.3 ± 3.3	72.2 ± 3.1	69.9 ± 0.7
22	95% O_2/5% CO_2	Control	68.6 ± 2.0	86.9 ± 0.9	72.2 ± 1.2	76.0 ± 1.4
22	95% O_2/5% CO_2	Morphine (30)	58.5 ± 2.0[a]	83.0 ± 1.4	71.4 ± 0.9	75.4 ± 2.3
37	Air	Control	61.0 ± 6.6	86.8 ± 2.9	71.4 ± 3.1	73.2 ± 2.8
37	Air	Morphine (30)	33.7 ± 2.2[a]	56.5 ± 8.0[a]	37.6 ± 4.4[a]	46.3 ± 0.6[a]

[a] Treatment different from respective control, Student's *t*-test, $p < 0.05$.

morphine varying in orders of magnitude from 10^{-5} to 10^{-3} M. The experiments depicted in Fig. 2A and B show that morphine, 10^{-5}–10^{-3} M, did not affect the rate of protein synthesis in either system. Thus, it is unlikely that the decreased rate of protein synthesis observed when morphine is administered to rats is due to a direct effect of the drug on the protein synthetic machinery; hence, the response in the intact animal is probably due to an effect of the drug on some physiological process.

Effects of treatments that mimic morphine action on physiological processes. Morphine is a potent respiratory depressant acting on medullary sites to decrease respiratory rate and produce acidosis and hypoxia (Table 4). Thus, as one approach to the analysis of the mechanism by which morphine reduces the rate of protein synthesis in liver, treatments that produce either acidosis or hypoxia were examined.

To investigate whether the decrease in the rate of protein synthesis might be due to systemic acidosis, rats which had been treated with acetazolamide (100 mg/kg) for 30 min were pulse-labeled with [^{3}H]leucine as above. Table 4 shows that acetazolamide produced a degree of acidosis and hypercapnia similar to that obtained with morphine (30 mg/kg) (line 4 compared with line 3). However, it did not affect either the rate of protein synthesis (Tables 1 and 2) or the content of polysomes in brain or liver (Table 3). Thus, it is unlikely that the decreased rate of protein synthesis observed when morphine is administered to rats is due to systemic acidosis; hence, hypoxia may be responsible for the inhibition of hepatic protein synthesis.

To test this hypothesis, rats which had been exposed to an atmosphere of 10% O_2/90% N_2 for 30 min were pulse-labeled with [^{3}H]leucine under the same conditions. Table 4 shows that this treatment produced a degree of hypoxia similar to that obtained with morphine (30 mg/kg). Moreover, it reduced the rate of protein synthesis in liver by 50% (Table 2), but did not affect the rate in brain (Table 1). In addition, it did not affect either the content of polysomes (Table 3) or the amount of intracellular radioactivity in brain or liver. However, it should be noted that hypoxia reduced completed protein release more than nascent chain synthesis, suggesting that termination rather than elongation was inhibited. Nevertheless, the results in this section indicate that hypoxia has a differential effect on hepatic protein synthesis, like morphine, even though the extent and site of inhibition appear to be somewhat different.

Effects of treatments that prevent hypoxia. The preceding analysis implicates hypoxia; it follows, therefore, that the inhibition of hepatic protein synthesis by morphine should be prevented by increasing arterial P_{O2}. Two independent approaches were used to test this hypothesis. In the first approach rats which had been treated with morphine (30 mg/kg) plus the opiate antagonist naloxone or with naloxone alone for 35 min were pulsed-labeled with [^{3}H]leucine. As expected, naloxone prevented the effects of morphine on both physiological factors (Table 4) and hepatic protein synthesis (Table 2). When administered alone, it did not affect any of the parameters studied here (Tables 1–4).

In the second approach, rats which had been treated with morphine (30 mg/kg) or 0.9% NaCl and exposed to an atmosphere of 95% O_2/5% CO_2 for 30 min were pulse-labeled with [^{3}H]leucine under the same conditions. Table 4 shows that there was no difference in arterial P_{O2}; however, both groups exhibited acidosis and hypercapnia. Nevertheless, morphine did not affect the rate of protein synthesis in either brain (Table 1) or liver (Table 2). The experiments in this section indicate that the

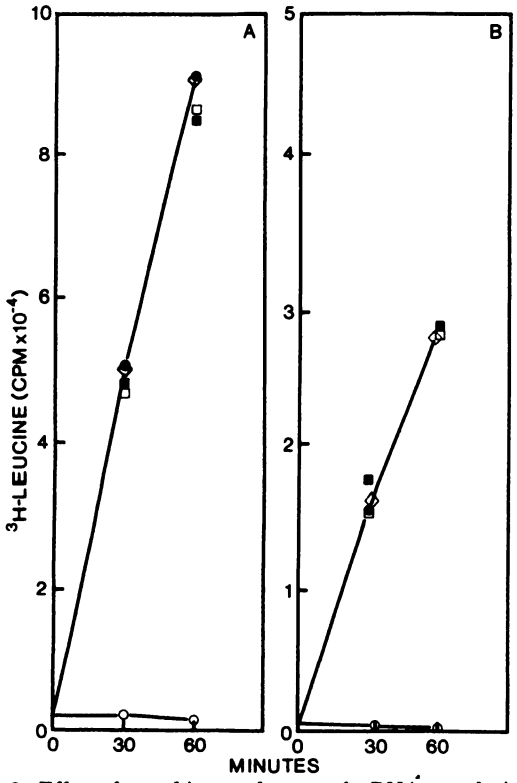

FIG. 2. *Effect of morphine on the rate of mRNA translation in cell-free systems derived from wheat germ embryos (A) and rabbit reticulocytes (B)*

Wheat germ embryo and rabbit reticulocyte lysate translation systems were incubated in the absence (◇) and presence of 10^{-5} M (■), 10^{-4} M (□), and 10^{-3} M (●) morphine with [^{3}H]leucine as the labeled amino acid and liver-free polysomal poly(A$^+$) mRNA as the source of mRNA; (○), blanks containing no exogenous mRNA. At the times indicated, aliquots were taken to measure total protein synthesis. The results are expressed as counts per minute per 5μl of reaction mixture and represent the mean values from three experiments. The standard error is within 4–8% of the indicated values at all time points.

inhibition of hepatic protein synthesis observed following acute morphine administration is due to hypoxia rather than a direct effect of morphine on the protein synthetic machinery.

Effects of morphine and other treatments on ATP concentration. Since energy production normally limits the rate of protein synthesis in hypoxic tissues, the concentration of ATP was determined following all treatments that either produced or prevented inhibition of hepatic protein synthesis. Table 5 shows that all treatments that produced inhibition (morphine, 30 mg/kg, and 10% O_2/90% N_2) reduced the concentration of ATP in liver by 50% but did not affect the concentration in brain, whereas all treatments that prevented inhibition (naloxone plus morphine and morphine plus 95% O_2/5% CO_2) did not affect the concentration of ATP in either tissue. Thus, it is the concentration of ATP that is rate-limiting for hepatic protein synthesis. Furthermore, since cerebral concentrations of ATP were unaltered by morphine-induced hypoxia, it seems likely that cerebral blood flow was increased (22, 23) to maintain the tissue in energy balance.

To test this hypothesis, rats which had been treated with morphine (30 mg/kg) or 0.9% NaCl and maintained in an ambient temperature of 37° for 30 min were pulse-labeled with [^{3}H]leucine under the same conditions, while two similar groups served for the analysis of adenylate nucleotides. We reasoned that if the difference in ATP concentration between brain and liver was due to differential rates of ATP regeneration, then the characteristic effects of morphine should be produced in brain as well as liver under conditions that would enhance the utilization of ATP, i.e., an ambient temperature of 37°.

Tables 1 and 2 show that morphine treatment in a hot environment reduced the rate of protein synthesis in brain by 60% and in liver by 95%. Moreover, Table 5 shows that this treatment reduced the concentration of

TABLE 4

Effects of morphine and other treatments on arterial blood pH, P_{CO_2} and P_{O_2}

Rats were treated as described in Table 1 and under Materials and Methods. Arterial blood samples were obtained immediately prior to sacrifice. Analyses are described under Materials and Methods. The values are the means ± standard error of determinations on four to eight animals.

Conditions			pH	P_{CO_2}	P_{O_2}
Environmental temperature	Atmosphere	Drug and dose (mg/kg)			
				mm Hg	*mm Hg*
22°	Air	Control	7.50 ± 0.02	37 ± 4	89 ± 4
22	Air	Morphine (10)	7.33 ± 0.02[a]	53 ± 4[a]	60 ± 7[a]
22	Air	Morphine (30)	7.18 ± 0.03[a]	60 ± 5[a]	48 ± 8[a]
22	Air	Acetazolamide (100)	7.32 ± 0.02[a]	54 ± 2[a]	86 ± 7
22	10% O_2/90% N_2	—	7.41 ± 0.01	31 ± 3	46 ± 2[a]
22	Air	Naloxone (6)	7.47 ± 0.06	33 ± 2	90 ± 5
22	Air	Naloxone (6) + morphine (30)	7.42 ± 0.02	40 ± 3	83 ± 6
22	95% O_2/5% CO_2	Control	7.36 ± 0.01	51 ± 4	252 ± 25
22	95% O_2/5% CO_2	Morphine (30)	7.03 ± 0.02[b]	111 ± 6[b]	243 ± 11
37	Air	Control	7.48 ± 0.01	35 ± 2	80 ± 4
37	Air	Morphine (30)	7.23 ± 0.01[b]	55 ± 1[b]	42 ± 2[b]

[a] Treatment different from respective control, one-way analysis of variance and Student-Newman-Keul's test, $p < 0.05$.
[b] Treatment different from respective control, Student's t-test, $p < 0.05$.

TABLE 5

Effects of morphine and other treatments on ATP concentration in rat liver and brain

Rats were treated as described in Table 1 and under Materials and Methods, except that nonradioactive leucine was used and perfusion was omitted. Analyses are described under Materials and Methods. The values are the means ± standard error of determinations on four to eight animals.

Conditions			Liver	Brain
Environmental temperature	Atmosphere	Drug and dose (mg/kg)		
			μmoles ATP/g tissue	*μmoles ATP/g tissue*
22°	Air	Control	2.59 ± 0.04	2.47 ± 0.05
22	Air	Morphine (30)	1.23 ± 0.05[a]	2.17 ± 0.11
22	10% O_2/90% N_2	—	1.20 ± 0.04[a]	2.41 ± 0.07
22	Air	Naloxone (6)	2.74 ± 0.02	2.39 ± 0.02
22	Air	Naloxone (6) + morphine (30)	2.36 ± 0.16	2.46 ± 0.22
22	95% O_2/5% CO_2	Control	2.46 ± 0.12	2.50 ± 0.28
22	95% O_2/5% CO_2	Morphine	2.33 ± 0.09	2.50 ± 0.11
37	Air	Control	2.56 ± 0.18	2.42 ± 0.08
37	Air	Morphine	0.98 ± 0.10[b]	1.88 ± 0.10[b]

[a] Treatment different from respective control, one-way analysis of variance and Student-Newman-Keul's test, $p < 0.05$.
[b] Treatment different from respective control, Student's t-test, $p < 0.05$.

ATP in brain by 20% and in liver by 60%. In contrast, morphine treatment in a hot environment did not potentiate the usual effects of morphine (30 mg/kg) on physiological factors (Table 4), nor did it affect the amount of intracellular radioactivity or the mode of inhibition of protein synthesis (Tables 1 and 2), even though it produced large decreases in the content of polysomes in both tissues (Table 3). Thus, the characteristic effects of morphine were produced in brain as well as liver by treatment with morphine in a hot environment. The decrease in ATP concentration in brain under these conditions, therefore, is at least partially due to an increased rate of ATP utilization, since if the major effect of hyperthermia were to decrease the rate of ATP regeneration, the concentration of ATP in hyperthermic controls should be lower than that in normothermic controls. Thus, we conclude that the differential effect of morphine on cerebral and hepatic protein synthesis in a normal environment is due to differential rates of ATP regeneration, probably resulting from hypoxia-induced enhancement of blood flow and oxygen/substrate supply to the brain.

The rate of protein synthesis in bacteria (24) and in mammalian cells in culture (25) has been shown to be much more sensitive to changes in the value of the adenylate energy charge than to changes in the concentrations of ATP. To ascertain whether this phenomenon is also observed *in vivo*, data from the previous studies on adenylate nucleotide concentrations were expressed as the adenylate energy charge (24) and plotted against the rate of protein synthesis (Fig. 3). In all cases, the rate of protein synthesis was more sensitive to changes in the value of the energy charge than to changes in the concentration of ATP. For example, the rate of protein synthesis in brain after treatment with morphine in a hot environment was 40% of normal when the concentration of ATP fell by 22% and when the energy charge fell by less than 9%.

DISCUSSION

Morphine in sufficient doses reduced the rate of protein synthesis in liver without affecting the rate in brain. This result was obtained whether the rates were measured 30, 60, 90, or 120 min after acute subcutaneous administration of morphine (30 mg/kg). The unresponsiveness of cerebral protein synthesis is therefore not a time-related phenomenon. Moreover, the degree of inhibition of hepatic protein synthesis was similar at all four time intervals. Since the pattern of hepatic responses to

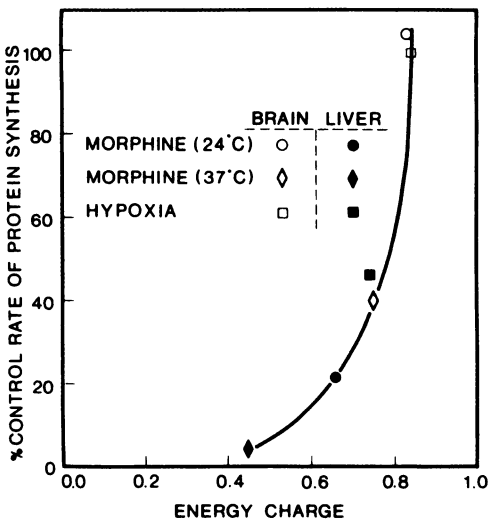

FIG. 3. *Relationship of the rate of protein synthesis in vivo in rat brain and liver to the adenylate energy change*

The ratio of the rate of protein synthesis in the treated rats to that in control rats is derived from the data in Tables 1 and 2 and expressed as a percentage. Adenylate nucleotides were determined in duplicate or triplicate assays as described under Materials and Methods, and the adenylate energy change (24) was calculated as the ratio of molar concentrations of (ATP + ½ ADP)/(ATP + ADP + AMP).

higher doses of morphine does not parallel that of hypothermic responses (26), it seems unlikely that hypothermia is a causative factor. This conclusion agrees with earlier findings (5).

The mechanism of inhibition of protein synthesis was analyzed by investigating both potential means of reducing the rate of protein synthesis, i.e., a decrease in the function of the protein synthetic machinery and a decrease in the concentration of some factor required for protein synthesis.

The first possibility was investigated by examining the rate of protein synthesis in two different cell-free systems in the presence and absence of morphine. No decrease in the rate of protein synthesis was observed when morphine was present in concentrations up to 10^{-3} M. These results suggest that the response in the intact animal is due to an effect of the drug on some physiological process.

Experiments were performed to determine whether the hepatic response results from acidosis or hypoxia, the most prominent systemic effects of higher doses of morphine. No decrease in the rate of protein synthesis was observed when acetazolamide was administered at a dose that produced a degree of acidosis and hypercapnia similar to that obtained with morphine. However, there was a decrease in the rate of protein synthesis when inspired O_2 concentration was reduced to a level that produced a degree of hypoxia similar to that obtained with morphine. Furthermore, no decrease in the rate of protein synthesis was observed when respiratory depression was prevented by concomitant administration of naloxone with morphine or when arterial P_{O2} was elevated in morphine-treated rats by increasing inspired O_2 concentration. Thus, it is the respiratory depression and resulting hypoxia that accounts for the inhibition of hepatic protein synthesis.

Effects of morphine on the concentration of factors required for protein synthesis were analyzed by measuring ATP concentrations. No decrease in liver ATP concentration was observed following any of the treatments that prevented inhibition of protein synthesis (naloxone plus morphine or morphine plus 95% O_2/5% CO_2). However, there was a decrease in the concentration of ATP in liver following both treatments that produced inhibition (morphine, 30 mg/kg, and 10% O_2/90% N_2). Thus, it is the concentration of ATP that is rate-limiting for hepatic protein synthesis, since it is generally recognized that protein synthesis is a highly endergonic process in the liver, consuming at least 50% of the energy supply, in contrast to the situation in the brain. On the other hand, no change in brain ATP concentration was ever observed after morphine or 10% O_2/90% N_2 administration, in agreement with findings of others (27). In order for cerebral concentrations of ATP to be maintained when arterial P_{O2} decreased, there must have been a compensatory increase in cerebral blood flow to cover the oxygen/substrate requirements of the tissue. This conclusion is consistent with results showing that both morphine (22) and hypoxia (23) produce increased cerebral blood flow and normal or elevated oxygen consumption. Thus, it is the increased cerebral blood flow that accounts for the differential effect of morphine on cerebral and hepatic protein synthesis.

One previous study examined the role of energy production in the mode of action of opiates as inhibitors of cell growth and protein synthesis. Greene and Magasanik (2), studying *Escherichia coli*, have concluded that opiates have no effect on energy production. However, their results are difficult to interpret owing to insufficient evidence to show that chloramphenicol-treated controls were, in fact, appropriate controls for this analysis.

In attempting to understand how energy flux affects protein synthesis in the intact animal, we have investigated the relationship between the rate of protein synthesis and the adenylate energy charge. Other workers have investigated this relationship in *E. coli* (24) and in ascites tumor cells (25). However, prior to this study clear correlations between the rate of protein synthesis and the adenylate energy charge have not been demonstrated in the intact animal. The results presented here suggest that the adenylate energy charge is the effective regulatory parameter of protein synthesis *in vivo*.

From the analysis of protein synthesis we conclude that morphine-induced hypoxia blocks protein synthesis by reducing the rate of polypeptide elongation. The maintenance of polysome size is consistent with this idea. The inhibition of protein synthesis in reticulocytes and ascites tumor cells by impairment of energy metabolism (28) and in rat brain by ischemia (29) is also characterized by the maintenance of polysome size. Moreover, the stability of isolated brain polysomes to incubation *in vitro* is enhanced by pretreatment of mice with high doses of morphine (30). However, in none of these cases has evidence been presented that ATP depletion produced these effects by reducing the rate of elongation.

The role of protein synthesis in the acute effects of morphine is clarified somewhat by these studies. In a room temperature environment, no decrease in the rate of protein synthesis in brain was ever observed when morphine, 10 or 30 mg/kg, was administered. Indeed, even in a hot environment, where morphine completely blocked hepatic protein synthesis, it reduced the rate in brain by only 60%. Thus, it seems unlikely that gross changes in the rate of protein synthesis in brain are required for the expression of the acute effects of morphine. If there are changes, they are either very small or, more likely, localized in regions which comprise only a small fraction of the brain. On the other hand, it is likely that acute effects of large doses of morphine on other tissues may be due, in part, to hypoxia-induced inhibition of ATP regeneration or protein synthesis, or both. This hypothesis can be tested in these systems.

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REFERENCES

1. Noteboom, W. D., and G. C. Mueller. Inhibition of protein and RNA synthesis in HeLa cells by levallorphan and levorphanol. *Mol. Pharmacol.* 2:534–562 (1966).
2. Greene, R., and M. Magasanik. The mode of action of levallorphan as an inhibitor of cell growth. *Mol. Pharmacol.* 3:453–472 (1967).

3. Noteboom, W. D., and G. C. Mueller. Inhibition of cell growth and the synthesis of ribonucleic acid and protein in HeLa cells by morphinans and related compounds. *Mol. Pharmacol.* **5**:38–48 (1969).
4. Clouet, D. H., and M. Ratner. The effect of the administration of morphine on the incorporation of [^{14}C]leucine into the proteins of rat brain *in vivo*. *Brain Res.* **4**:33–43 (1967).
5. Clouet, D. H., and M. Ratner. The effect of morphine administration on the incorporation of [^{14}C]leucine into protein in cell-free systems from rat brain and liver. *J. Neurochem.* **15**:17–33 (1968).
6. Clouet, D. H., and M. Ratner. The effect of morphine on the transport and metabolism of intracisternally-injected leucine in the rat. *J. Neurochem.* **17**:1069–1074 (1970).
7. Kuschinsky, K. Effect of morphine on protein synthesis in synaptosomes and mitochondria of mouse brain *in vivo*. *Naunyn-Schmiedebergs Arch. Pharmakol.* **271**:294–300 (1971).
8. Harris, R. A., A. Dunn, and L. S. Harris. Effects of acute and chronic morphine administration on the incorporation of [^{3}H]lysine into mouse brain and liver proteins. *Res. Commun. Chem. Pathol. Pharmacol.* **9**:299–306 (1974).
9. Loh, H. H., and R. Hitzemann. Effect of morphine on the turnover and synthesis of (Leu-^{3}H) protein and (Ch-^{14}C)-phosphatidylcholine in discrete regions of the rat brain. *Biochem. Pharmacol.* **23**:1753–1765 (1974).
10. Nakajima, T., H. Sasano, M. Koida, and H. Kaneto. Protein synthesis in mouse brain during development of acute morphine tolerance. *Jpn. J. Pharmacol.* **25**:367–374 (1975).
11. Ratner, M., and D. H. Clouet. The effect of morphine on ^{14}C-leucine incorporation by rat liver microsomes *in vitro*. *Biochem. Pharmacol.* **13**:1655–1661 (1964).
12. Ramsey, J. C., and W. J. Steele. Differential effects of morphine on rates of translation *in vivo* in free and bound polysome compartments of rat brain regions, in *Opiates and Endogenous Opioid Peptides*. Elsevier, Amsterdam, 435–438 (1976).
13. Ramsey, J. C., and W. J. Steele. Quantitative isolation and properties of nearly homogenous populations of undegraded free and bound polysomes from rat brain. *J. Neurochem.* **28**:517–527 (1977).
14. Ramsey, J. C., and W. J. Steele. Differences in size, structure and function of free and membrane-bound polyribosomes of rat liver. *Biochem. J.* **168**:1–8 (1977).
15. Mans, R. J., and G. D. Novelli. Measurement of the incorporation of radioactive amino acids into protein by a filter-paper disk method. *Arch. Biochem. Biophys.* **94**:48–53 (1961).
16. Ramsey, J. C., and W. J. Steele. A procedure for the quantitative recovery of homogeneous population of undegraded free and bound polysomes from rat liver. *Biochemistry* **15**:1704–1712 (1976).
17. Ramsey, J. C., and W. J. Steele. A method for visualizing and quantifying the total complement of free and membrane-bound ribosomes in rat liver. *Anal. Biochem.* **92**:305–313 (1979).
18. Pelham, H. R. B., and R. J. Jackson. An efficient mRNA-dependent translation system from reticulocyte lysates. *Eur. J. Biochem.* **67**:247–256 (1976).
19. Marcu, K., and B. Dudock. Characterization of a highly efficient protein synthesizing system derived from commercial wheat germ. *Nucleic Acids Res.* **1**:1385–1397 (1974).
20. Wollenberger, A., O. Ristau, and G. Schoffa. Eine einfache Technik der extrem Schnellen abkuhlung grosserer Gewebestucke. *Eur. J. Physiol.* **270**:399–412 (1960).
21. Kimmich, G. A., J. Randles, and J. S. Brand. Assay of picomole amounts of ATP, ADP and AMP using the luciferase enzyme system. *Anal. Biochem.* **69**:187–206 (1975).
22. Moyer, J. H., R. Pontius, G. Morris, and R. Hershberger. Effect of morphine and N-allylnormorphine on cerebral hemodynamics and oxygen metabolism. *Circulation* **15**:379–384 (1957).
23. Berntman, L., and B. K. Siesjö. Cerebral metabolic and circulatory changes induced by hypoxia in starved rats. *J. Neurochem.* **31**:1265–1276 (1978).
24. Swedes, J. S., R. J. Sedo, and D. E. Atkinson. Relation in growth and protein synthesis to the adenylate energy charge in an adenine-requiring mutant of *Escherichia coli*. *J. Biol. Chem.* **250**:6930–6938 (1975).
25. Live, T. R., and E. Kaminskas. Changes in adenylate energy charge in Ehrlich ascites tumor cells deprived of serum, glucose or amino acids. *J. Biol. Chem.* **250**:1786–1789 (1975).
26. McDougal, J. N., P. R. Marques, and T. F. Burks. Age-related changes in body temperature responses to morphine in rats. *Life Sci.* **27**:2679–2685 (1980).
27. Miller, A. L., R. A. Hawkins, R. L. Harris, and R. L. Veech. The effects of acute and chronic morphine treatment and of morphine withdrawal on rat brain *in vivo*. *Biochem. J.* **129**:463–469 (1972).
28. Freudenberg, H., and J. Mager. Studies on the mechanism of the inhibition of protein synthesis induced by intracellular ATP depletion. *Biochim. Biophys. Acta* **232**:537–555 (1971).
29. Cooper, H. K., T. Zalewska, S. Kawakami, K.-A. Hossmann, and P. Kleihues. The effect of ischaemia and recirculation on protein synthesis in the rat brain. *J. Neurochem.* **28**:929–934 (1977).
30. Stolman, S., and H. H. Loh. Stabilization of brain free polysomes by morphine. *Res. Commun. Chem. Pathol. Pharmacol.* **12**:419–424 (1975).

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