The Effects of Acute and Chronic Morphine Treatment and of Morphine Withdrawal on Rat Brain *in vivo*

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1. The effects of morphine, nalorphine, acetazolamide, and 10% CO₂ on brain metabolite concentrations of 24h-starved rats were studied. 2. A single dose of morphine (20mg/kg body wt.) caused an increase in brain glucose concentration (42%) and decreased concentrations of lactate (24%), pyruvate (29%), citrate (20%), α -oxoglutarate (16%), malate (14%) and creatine phosphate (10%) after 30min. No changes were found in adenine nucleotide concentrations. 3. The same dose of morphine increased arterial CO₂ from 5.07 to 7.60 kN/m² (38 to 57 Torr), decreased the pH from 7.41 to 7.31 and decreased O₂ from 14.1 to 10.8 kN/m² (106 to 81 Torr) at 30min. 4. Rats injected with morphine three times daily (20mg/kg body wt.) for 2 weeks had no changes in brain metabolite concentrations or in blood gases 30min after their last injection. 5. Nalorphine (an antagonist of morphine) caused essentially no changes in brain metabolite concentrations in normal rats. When nalorphine (20mg/kg) was administered to rats previously treated with morphine three times daily for 2 weeks, there was an increase in brain glucose (100%), lactate (23%), pyruvate (18%) and citrate (10%) concentrations. 6. Acetazolamide (an inhibitor of carbonic anhydrase) and 10% CO₂ increased the arterial CO₂ from 4.79 to 6.78 kN/m² (36 to 51 Torr) and from 5.32 to 10.8 kN/m² (40 to 81 Torr) respectively. 7. Both acetazolamide and 10% CO₂ caused changes in brain metabolite concentrations similar to those for acutely administered morphine. Thus 10% CO₂ caused increased brain glucose concentration (123%) and decreased brain lactate (46%), pyruvate (34%), citrate (26%), α -oxoglutarate (33%), malate (45%) and creatine phosphate (7%) concentrations. No changes in adenine nucleotide concentrations were found. 8. The results indicate that the effect of morphine on brain metabolite concentrations may be accounted for by the increased [CO₂]. 9. These findings constitute a consistent pattern of metabolic changes after acute morphine administration, morphine addiction, and withdrawal from morphine addiction.

Previous studies of the effect of morphine *in vivo* have found changes in brain concentrations of individual metabolites, but no cause for these changes has been clearly identified. Morphine has been reported to increase rat brain ATP concentrations (Abood *et al.*, 1950; Estler & Ammon, 1964; Nichols *et al.*, 1970), decrease lactate and creatine phosphate concentrations in mouse brain (Estler & Heim, 1962), and to decrease the incorporation of carbon from labelled glucose into γ -aminobutyrate and aspartate (Bachelard & Lindsay, 1966). Dodge & Takemori (1972) reported that single doses of morphine increased rat brain glucose, glucose 6-phosphate, fructose 6-phosphate, creatine phosphate and ATP concentrations, whereas pyruvate and lactate concentrations were unchanged. Most of these changes were either absent or were less marked in rats chronically treated with morphine, including a group given nalorphine to induce the withdrawal syndrome.

The interpretation of previous studies of rat brain metabolism *in vivo* has been hindered by post-mor-

tem changes in brain metabolite concentrations (Swaab, 1971). With the development of an apparatus for the rapid removal and freezing of rat brain (Veech *et al.*, 1972), these problems have been overcome.

Initial experiments showed that morphine lowered the concentrations of many intermediates of glycolysis and the tricarboxylic acid cycle. However, morphine also caused respiratory depression with a concomitant increase in blood [CO₂]. In view of this, the relationship between the effects of morphine and of CO₂ was explored. It was found that the changes produced by morphine could be accounted for by the increased concentrations of CO₂.

Materials and Methods

Rats

Male albino rats of the Wistar-Furth strain (Carruth, New York, N.Y., U.S.A.) weighing 200–250g were used. All rats were starved for 24h before use.

Reagents

Enzymes and coenzymes were obtained from Boehringer (Mannheim) Corp., New York, N.Y., U.S.A. Morphine sulphate was obtained from S. B. Penick, New York, N.Y., U.S.A.; *N*-allylnormorphine hydrochloride (nalorphine) was from Merck, Sharpe and Dohme, West Point, Pa., U.S.A.; 2-acetyl-amino-1,3,4-thiadiazole-5-sulphonamide (acetazolamide) was from Lederle Laboratories, Pearl River, N.Y., U.S.A.; and a mixture of $\text{CO}_2 + \text{O}_2 + \text{N}_2$ (10:21:69) was from Air Products Inc., Emmaus, Pa., U.S.A.

Analytical methods

The metabolites were determined by standard enzymic techniques as follows: lactate (Hohorst, 1963b); pyruvate (Bücher *et al.*, 1963); α -oxoglutarate (Bergmeyer & Bernt, 1963); malate (Hohorst, 1963c); citrate (Moellering & Gruber, 1966); glucose 6-phosphate (Hohorst, 1963a); glutamate (Bernt & Bergmeyer, 1963); ATP (Lamprecht & Trautschold, 1963); ADP (Adam, 1963); creatine phosphate and creatine (Lowry *et al.*, 1964); aspartate (Pfleiderer, 1963); glucose (Slein, 1963); ammonia (Folbergrova *et al.*, 1969). Lactate and malate were determined sequentially in the same cuvette, as were pyruvate and α -oxoglutarate, by the addition of the appropriate enzymes. Adenine nucleotides, creatine phosphate, ammonia, pyruvate and α -oxoglutarate were measured immediately after extraction. The remaining extract was frozen and the other metabolites were measured within 3 days. All metabolite concentrations are expressed as $\mu\text{mol/g}$ fresh wt. of brain.

Removal and treatment of brain

The supratentorial portion of the brain was removed and frozen within 1 s by using the rapid-freezing apparatus (Precision Medical Instruments, Shalimar, Fla., U.S.A.) described by Veech *et al.* (1972). The brain sample (0.7–1.0 g) was ground under liquid N_2 , extracted in 6 ml of iced 8% (w/v) HClO_4 and neutralized with KOH to pH 6–7. Thereafter the samples were treated in the standard way.

Treatment of animals

For acute experiments morphine, nalorphine and acetazolamide were freshly dissolved in 0.9% NaCl and injected intraperitoneally at the times and dosages indicated. CO_2 was administered to rats positioned on the apparatus with the aid of a small chamber fitted to the machine, thus assuring minimal time (2–3 s) between cessation of CO_2 exposure and brain removal. For some experiments rats were chronically treated with morphine by intraperitoneal

injections (20 mg/kg body wt.) three times daily for 2 weeks. Morphine was injected at 09.00, 16.00 and 23.00 h each day. Control rats were injected with an equivalent volume of 0.9% NaCl.

Determination of blood gases and pH

Arterial blood was obtained from the femoral artery as described by Hawkins *et al.* (1971). Blood gases and pH were measured at 38°C in a 0.4 ml sample by using a combined pH-meter and gas monitor (Radiometer, Copenhagen, Denmark).

Statistical analyses

The statistical significances of the differences between mean metabolite concentrations of the control and experimental groups were calculated by using Student's *t* test. Because of the small sizes of the groups used for blood gas and pH measurements, the Mann-Whitney U test was used, with each group serving as its own control.

Results

Effects of morphine, acetazolamide and 10% CO_2 on blood gases and pH

Single injections of morphine caused CO_2 pressure to increase and O_2 pressure and pH to decrease within 10 min (Table 1). These changes were sustained for 90 min. Chronically morphine-treated rats showed no changes in these parameters, indicating the development of tolerance.

Results are included showing the changes caused by two other agents which increase CO_2 concentrations by different mechanisms. The direct effect of 10% CO_2 was compared with that of acetazolamide, an inhibitor of carbonic anhydrase (Mudge, 1966). Both of these agents caused an increase in O_2 pressure owing to hyperventilation, in addition to the expected increases in CO_2 pressures.

Effects of morphine and nalorphine on brain metabolite concentrations

Normal rats given morphine had increased brain concentrations of glucose (30–60%) and decreased concentrations of pyruvate (10–30%), lactate (22–25%) and malate (13–17%) after 10, 30 and 90 min (Table 2). Decreases also occurred in the concentrations of α -oxoglutarate (11–14%), citrate (14–20%) and creatine phosphate (10–21%) at 30–90 min.

Rats treated with morphine for 2 weeks (see the Materials and Methods section) had no changes in brain metabolite concentrations 30 min after the final injection (Table 3).

Table 1. Blood gases and pH after administration of morphine, acetazolamide and CO₂

The results are mean values ($\pm$ S.E.M.) with the number of observations in parentheses. The symbol * indicates statistical significance at the 5% level or below. Rats were injected with morphine sulphate (20 mg/kg body wt.), acetazolamide (100 mg/kg body wt.) or an equivalent amount of 0.9% NaCl intraperitoneally. Rats injected with both acetazolamide and morphine received the morphine 20 min after the acetazolamide and samples were taken before treatment and at 10 and 30 min after morphine injection. CO₂-treated rats were given a mixture of CO₂ + O₂ + N₂ (10:21:69). Chronically morphine-treated rats were injected with morphine (20 mg/kg body wt.) three times daily for 2 weeks. Blood was sampled from the femoral artery. For other experimental details see the Materials and Methods section.

Treatment	Time after treatment (min)	0	10	30	90
Morphine injected (3)	CO ₂ [kN/m ² (Torr)]	5.02 $\pm$ 0.04 (37.7 $\pm$ 0.3)	7.41 $\pm$ 0.63 (55.7 $\pm$ 4.7)*	7.62 $\pm$ 0.47 (57.3 $\pm$ 3.5)*	7.14 $\pm$ 0.63 (53.7 $\pm$ 4.7)*
	pH	7.41 $\pm$ 0.01	7.34 $\pm$ 0.02*	7.31 $\pm$ 0.03*	7.35 $\pm$ 0.04
	O ₂ [kN/m ² (Torr)]	14.1 $\pm$ 0.2 (106 $\pm$ 1.2)	12.1 $\pm$ 0.9 (91.0 $\pm$ 6.5)*	10.8 $\pm$ 0.5 (81.3 $\pm$ 4.1)*	11.0 $\pm$ 1.1 (82.7 $\pm$ 8.4)*
Chronically morphine injected (4)	CO ₂ [kN/m ² (Torr)]	4.79 $\pm$ 0.08 (36.0 $\pm$ 0.6)	5.55 $\pm$ 0.63 (41.7 $\pm$ 4.7)	5.29 $\pm$ 0.65 (39.8 $\pm$ 4.9)	5.12 $\pm$ 0.36 (38.5 $\pm$ 2.7)
	pH	7.44 $\pm$ 0.02	7.34 $\pm$ 0.07	7.34 $\pm$ 0.07	7.32 $\pm$ 0.04*
	O ₂ [kN/m ² (Torr)]	14.1 $\pm$ 0.2 (106 $\pm$ 1.5)	12.4 $\pm$ 0.9 (93.0 $\pm$ 7.0)	12.9 $\pm$ 0.47 (97.2 $\pm$ 3.5)	12.7 $\pm$ 0.4 (95.5 $\pm$ 3.3)
Acetazolamide injected (4)	CO ₂ [kN/m ² (Torr)]	4.79 $\pm$ 0.15 (36.0 $\pm$ 1.1)	6.25 $\pm$ 0.23 (47.0 $\pm$ 1.7)*	6.76 $\pm$ 0.12 (50.8 $\pm$ 0.9)*	6.74 $\pm$ 0.36 (50.7 $\pm$ 2.7)*
	pH	7.38 $\pm$ 0.02	7.32 $\pm$ 0.01	7.23 $\pm$ 0.02*	7.19 $\pm$ 0.01*
	O ₂ [kN/m ² (Torr)]	12.9 $\pm$ 0.6 (97.2 $\pm$ 4.2)	16.8 $\pm$ 0.2 (126 $\pm$ 1.8)*	16.2 $\pm$ 0.7 (122 $\pm$ 5.0)*	17.4 $\pm$ 0.2 (131 $\pm$ 1.8)*
CO ₂ treated (4)	CO ₂ [kN/m ² (Torr)]	5.37 $\pm$ 0.17 (40.4 $\pm$ 1.3)	10.8 $\pm$ 0.2 (81.2 $\pm$ 1.3)*	11.4 $\pm$ 0.3 (85.8 $\pm$ 2.6)*	—
	pH	7.38 $\pm$ 0.003	7.14 $\pm$ 0.02*	7.12 $\pm$ 0.01*	—
	O ₂ [kN/m ² (Torr)]	13.8 $\pm$ 0.2 (104 $\pm$ 1.5)	20.2 $\pm$ 0.3 (152 $\pm$ 2.3)*	21.3 $\pm$ 0.08 (160 $\pm$ 0.6)*	—
Acetazolamide and morphine injected (3)	CO ₂ [kN/m ² (Torr)]	5.14 $\pm$ 0.15 (38.6 $\pm$ 1.1)	7.98 $\pm$ 0.81 (60.0 $\pm$ 6.1)*	9.35 $\pm$ 0.18 (70.3 $\pm$ 1.5)*	—
	pH	7.40 $\pm$ 0.01	7.19 $\pm$ 0.02*	7.12 $\pm$ 0.01*	—
	O ₂ [kN/m ² (Torr)]	13.4 $\pm$ 0.5 (101 $\pm$ 4)	15.4 $\pm$ 1.7 (116 $\pm$ 13)	13.8 $\pm$ 1.1 (104 $\pm$ 8)	—
NaCl injected (3)	CO ₂ [kN/m ² (Torr)]	5.81 $\pm$ 0.12 (43.7 $\pm$ 0.9)	5.36 $\pm$ 0.24 (40.3 $\pm$ 1.8)	5.63 $\pm$ 0.24 (42.3 $\pm$ 1.8)	5.59 $\pm$ 0.40 (42.0 $\pm$ 3.0)
	pH	7.42 $\pm$ 0.02	7.47 $\pm$ 0.01*	7.43 $\pm$ 0.01	7.45 $\pm$ 0.02
	O ₂ [kN/m ² (Torr)]	13.7 $\pm$ 0.1 (103 $\pm$ 0.9)	14.0 $\pm$ 0.1 (105 $\pm$ 0.9)	14.1 $\pm$ 0.3 (106 $\pm$ 2.5)	13.7 $\pm$ 0.3 (103 $\pm$ 2.0)

Normal rats injected with nalorphine (an antagonist of morphine) showed no changes in brain metabolites, with the exception of an increased glucose concentration (Table 3). When given nalorphine, rats chronically treated with morphine had some changes in the opposite direction to those caused by single doses of morphine. Increased brain concentrations of pyruvate (18%), lactate (23%) and citrate (11%) occurred 30 min after the last morphine injection (20 min after nalorphine) (Table 3).

Effects of agents that increase blood [CO₂] on rat-brain metabolite concentrations

Since acutely administered morphine altered both brain metabolite concentrations and blood [CO₂], whereas chronic administration of morphine caused no changes in either, the effects of elevating [CO₂] by other means on brain metabolite concentrations were examined (Table 4). The patterns of changes in metabolite concentrations observed after administration of acetazolamide and 10% CO₂ were nearly identical with those observed after acute administration of morphine (Table 2). Morphine and acetazolamide together produced larger changes in metabolite concentrations than either given alone, in agreement with their additive effect on blood [CO₂] (Table 1).

Redox changes after morphine and CO₂ treatment

Small decreases in the [lactate]/[pyruvate] ratio were observed 10 min after administration of morphine and CO₂ (Table 5). The ratio returned to the control value 30 min after morphine injection.

The increases in [CO₂] were reflected in an increase in the [NADP⁺]/[NADPH] ratios whether calculated from the reactants of isocitrate dehydrogenase (EC 1.1.1.42) or 'malic' enzyme (EC 1.1.1.40). The increase in the free [NADP⁺]/[NADPH] ratio could be accounted for entirely by the increase in [CO₂].

Brain tissue pH

It was expected that intracellular [H⁺] would increase as [CO₂] increased. The reactants of the creatine kinase reaction (EC 2.7.3.2) have been used as an indicator of intracellular pH (Kuby & Noltman, 1962; Rose, 1968; Collins *et al.*, 1970). The changes in the ratio [ATP][creatine]/[ADP][creatine phosphate] calculated from the results in Tables 2 and 4 were consistent with an increase in [H⁺] of 15–20% at 30 and 90 min after morphine injection or after 10 min in a 10% CO₂ atmosphere. The change in [H⁺] corresponded to a decrease in pH of 0.065–0.080 unit.

Since [H⁺] is a net reactant in the lactate dehydrogenase reaction (EC 1.1.1.27) the ratio of free cytoplasmic [NAD⁺]/[NADH] was not calculated from

Table 2. *Effect of morphine on metabolite concentrations in rat brain*

The results are mean values ($\pm$ S.E.M.) with the number of observations in each group in parentheses. The symbols * and ** indicate statistical significance at the 5 and 1% levels respectively. Rats were injected with morphine sulphate (20mg/kg body wt.) or an equivalent volume of 0.9% NaCl intraperitoneally and killed at the indicated times after injection. Since there was no difference between control groups 10, 30 and 90min after injection of 0.9% NaCl they were combined. For other experimental details see the Materials and Methods section.

Metabolite	Time after injection ...	Concn. of metabolite (μ mol/g)			
		Control (32)	Morphine (10) 10min	Morphine (12) 30min	Morphine (12) 90min
Glucose		1.02 $\pm$ 0.05	1.33 $\pm$ 0.07**	1.45 $\pm$ 0.15**	1.61 $\pm$ 0.18**
Glucose 6-phosphate		0.131 $\pm$ 0.004	0.124 $\pm$ 0.008	0.134 $\pm$ 0.004	0.134 $\pm$ 0.006
Pyruvate		0.118 $\pm$ 0.003	0.105 $\pm$ 0.006*	0.084 $\pm$ 0.002**	0.092 $\pm$ 0.002**
Lactate		1.51 $\pm$ 0.04	1.18 $\pm$ 0.06**	1.15 $\pm$ 0.04**	1.14 $\pm$ 0.06**
Citrate		0.305 $\pm$ 0.008	0.276 $\pm$ 0.006	0.244 $\pm$ 0.007**	0.261 $\pm$ 0.006**
α -Oxoglutarate		0.216 $\pm$ 0.005	0.205 $\pm$ 0.008	0.181 $\pm$ 0.005**	0.193 $\pm$ 0.006*
Malate		0.293 $\pm$ 0.009	0.256 $\pm$ 0.011*	0.253 $\pm$ 0.012*	0.244 $\pm$ 0.013**
Glutamate		10.8 $\pm$ 0.2	10.8 $\pm$ 0.1	10.9 $\pm$ 0.2	10.7 $\pm$ 0.3
Ammonia		0.296 $\pm$ 0.011	0.257 $\pm$ 0.014*	0.297 $\pm$ 0.010	0.332 $\pm$ 0.019
Aspartate		2.91 $\pm$ 0.05	2.95 $\pm$ 0.05	3.03 $\pm$ 0.10	2.63 $\pm$ 0.10**
ATP		2.25 $\pm$ 0.03	2.28 $\pm$ 0.03	2.18 $\pm$ 0.04	2.00 $\pm$ 0.06**
ADP		0.707 $\pm$ 0.026	0.741 $\pm$ 0.034	0.685 $\pm$ 0.017	0.697 $\pm$ 0.028
Creatine phosphate		3.54 $\pm$ 0.06	3.41 $\pm$ 0.06	3.19 $\pm$ 0.07**	2.80 $\pm$ 0.09**
Creatine		5.42 $\pm$ 0.21 (9)	—	5.81 $\pm$ 0.20 (5)	5.56 $\pm$ 0.14 (10)

Table 3. *Effects of morphine and nalorphine on brain metabolite concentrations of chronically morphine-treated rats*

The results are mean values ($\pm$ S.E.M.) with the number of observations in each group in parentheses. The symbol * indicates statistical significance at the 5% level as compared with NaCl controls, and the symbols † and ‡ indicate statistical significance at the 5 and 1% levels respectively compared with morphine-treated rats. Rats were injected intraperitoneally with morphine sulphate (20mg/kg body wt.) or an equivalent volume of 0.9% NaCl three times daily for 2 weeks. They were killed 30 min after the final injection of NaCl or morphine. Nalorphine hydrochloride (20mg/kg body wt.) was injected 10 min after NaCl or morphine and 20 min before death. For other experimental details see Table 1.

Metabolite	Chronic treatments ... Last injections ...	Concn. of metabolite (μ mol/g)			
		NaCl NaCl (6)	Morphine Morphine (8)	NaCl Nalorphine (8)	Morphine Morphine and nalorphine (7)
Glucose		0.929 $\pm$ 0.094	1.03 $\pm$ 0.05	1.15 $\pm$ 0.05*	1.88 $\pm$ 0.22‡
Glucose 6-phosphate		0.116 $\pm$ 0.009	0.126 $\pm$ 0.004	0.107 $\pm$ 0.009	0.128 $\pm$ 0.005
Pyruvate		0.103 $\pm$ 0.004	0.099 $\pm$ 0.006	0.103 $\pm$ 0.003	0.122 $\pm$ 0.008†
Lactate		1.49 $\pm$ 0.11	1.35 $\pm$ 0.06	1.40 $\pm$ 0.07	1.83 $\pm$ 0.11‡
Citrate		0.300 $\pm$ 0.007	0.286 $\pm$ 0.009	0.309 $\pm$ 0.012	0.332 $\pm$ 0.018†
α -Oxoglutarate		0.212 $\pm$ 0.012	0.219 $\pm$ 0.010	0.205 $\pm$ 0.009	0.228 $\pm$ 0.009
Malate		0.287 $\pm$ 0.024	0.313 $\pm$ 0.015	0.280 $\pm$ 0.007	0.340 $\pm$ 0.022
Glutamate		10.4 $\pm$ 0.2	10.5 $\pm$ 0.3	10.6 $\pm$ 0.2	10.6 $\pm$ 0.5
Ammonia		0.275 $\pm$ 0.037	0.332 $\pm$ 0.029	0.244 $\pm$ 0.016	0.309 $\pm$ 0.034
Aspartate		2.64 $\pm$ 0.12	2.66 $\pm$ 0.07	2.78 $\pm$ 0.07	2.68 $\pm$ 0.15
ATP		2.15 $\pm$ 0.05	2.11 $\pm$ 0.05	2.18 $\pm$ 0.04	2.18 $\pm$ 0.08
ADP		0.695 $\pm$ 0.043	0.737 $\pm$ 0.025	0.728 $\pm$ 0.034	0.677 $\pm$ 0.044
Creatine phosphate		3.34 $\pm$ 0.06	3.13 $\pm$ 0.11	3.36 $\pm$ 0.09	3.31 $\pm$ 0.13

Table 4. *Effect of agents that elevate blood [CO₂] on rat brain metabolite concentrations*

The results are mean values ($\pm$ S.E.M.) with the number of observations in each group in parentheses. The symbols * and ** indicate statistical significance at the 5 and 1% levels respectively. Rats were injected intraperitoneally with acetazolamide (100mg/kg body wt.) 30min before being killed and with morphine sulphate (20mg/kg body wt.) 10min before being killed. The 10% CO₂ was administered for a period of 10min. The control values were combined. For other details see the Materials and Methods section and Table 1.

Metabolite	Concn. of metabolite (μ mol/g)			
	Control (13)	Acetazolamide (6)	Acetazolamide and morphine (6)	10% CO ₂ (7)
Glucose	1.03 $\pm$ 0.07	1.42 $\pm$ 0.07**	1.55 $\pm$ 0.07**	2.30 $\pm$ 0.10**
Glucose 6-phosphate	0.143 $\pm$ 0.005	0.137 $\pm$ 0.006	0.122 $\pm$ 0.006*	0.142 $\pm$ 0.005
Pyruvate	0.103 $\pm$ 0.003	0.079 $\pm$ 0.003**	0.051 $\pm$ 0.005**	0.068 $\pm$ 0.003**
Lactate	1.37 $\pm$ 0.06	0.932 $\pm$ 0.045**	0.599 $\pm$ 0.050**	0.631 $\pm$ 0.035**
Citrate	0.323 $\pm$ 0.012	0.257 $\pm$ 0.011**	0.254 $\pm$ 0.022**	0.240 $\pm$ 0.005**
α -Oxoglutarate	0.215 $\pm$ 0.006	0.155 $\pm$ 0.005**	0.134 $\pm$ 0.007**	0.145 $\pm$ 0.007**
Malate	0.261 $\pm$ 0.010	0.169 $\pm$ 0.008**	0.157 $\pm$ 0.011**	0.144 $\pm$ 0.010**
Glutamate	11.7 $\pm$ 0.1	10.7 $\pm$ 0.2**	9.6 $\pm$ 0.2**	11.0 $\pm$ 0.4
Ammonia	0.360 $\pm$ 0.026	0.336 $\pm$ 0.024	0.312 $\pm$ 0.018	0.309 $\pm$ 0.028
Aspartate	2.67 $\pm$ 0.05	2.57 $\pm$ 0.05	2.43 $\pm$ 0.07*	3.33 $\pm$ 0.09**
ATP	2.25 $\pm$ 0.03	2.30 $\pm$ 0.04	2.16 $\pm$ 0.05	2.19 $\pm$ 0.06
ADP	0.644 $\pm$ 0.030	0.688 $\pm$ 0.018	0.600 $\pm$ 0.022	0.569 $\pm$ 0.014
Creatine phosphate	3.49 $\pm$ 0.06	3.30 $\pm$ 0.05*	2.97 $\pm$ 0.10**	3.23 $\pm$ 0.11*
Creatine	4.99 $\pm$ 0.10			4.94 $\pm$ 0.13

Table 5. *[Lactate]/[pyruvate] and [NADP⁺]/[NADPH] ratios after administration of morphine and CO₂*

The values are means ($\pm$ S.E.M.) calculated from individual metabolite concentrations from experiments contained in Tables 1–4. The numbers in parentheses indicate the number of observations and the symbol ** indicates statistical significance at the 1% level. [NADP⁺]/[NADPH] ratios were calculated from the reactants of 'malic' enzyme, [NADP⁺]/[NADPH] = [pyruvate][CO₂]/[malate]K, and isocitrate dehydrogenase, [NADP⁺]/[NADPH] = [α -oxoglutarate][CO₂]/[isocitrate]K, where the equilibrium constants (K) were 3.44×10^{-2} M and 1.17 M respectively (Krebs & Veech, 1969; Londesborough & Dalziel, 1968). The concentration of isocitrate was assumed to be 5% that of citrate (Goldberg *et al.*, 1966). Brain tissue CO₂ concentrations (μ mol/g) were calculated from tissue P_{CO_2} as described by Krebs & Veech (1969). The value in the control animals was 1.19. Tissue P_{CO_2} was assumed to be equal to venous P_{CO_2} , which was taken to be 1.33 kN/m² (10 Torr) greater than arterial CO₂ pressures.

Ratio Time after treatment ...	Control (45)	Morphine (10) 10min	Morphine (12) 30min	Morphine (12) 90min	10% CO ₂ (7) 10min
[Lactate]/[pyruvate]	13.1 $\pm$ 0.3	11.4 $\pm$ 0.5**	13.7 $\pm$ 0.3	12.5 $\pm$ 0.5	9.35 $\pm$ 0.39**
$10^2 \times$ [NADP ⁺]/[NADPH] (‘malic’ enzyme)	1.41 $\pm$ 0.04	1.93 $\pm$ 0.09**	1.66 $\pm$ 0.10**	1.76 $\pm$ 0.09**	3.13 $\pm$ 0.19**
$10^2 \times$ [NADP ⁺]/[NADPH] (isocitrate dehydrogenase)	1.44 $\pm$ 0.03	2.03 $\pm$ 0.04**	2.10 $\pm$ 0.05**	1.99 $\pm$ 0.03**	2.62 $\pm$ 0.06**

the [lactate]/[pyruvate] ratio. Calculation of the free cytoplasmic [NADP⁺]/[NADPH] ratio is not affected by changes in [H⁺], since [H⁺] is not a net reactant in the systems used (Krebs & Veech, 1969).

Discussion

The results show that acute morphine administra-

tion caused increased CO₂ concentration and that the pattern of changes in metabolite concentrations observed after acute morphine injection could be reproduced by elevating CO₂ concentration by other means. The development of tolerance to morphine was accompanied by the disappearance of the changes in brain metabolite and CO₂ concentrations after morphine injection. The morphine antagonist nalorphine,

when administered to addicted rats, produced many changes in metabolite concentrations opposite to those found with acute morphine treatment. Taken together these results constitute a consistent pattern of metabolic changes in brain after acute morphine administration, morphine addiction and withdrawal of morphine. These results are in disagreement with those of Dodge & Takemori (1972), who reported no differences after nalorphine-induced withdrawal. This discrepancy is most probably due to the slower rate of brain freezing used by those authors. Significant post-mortem changes in metabolite concentrations occur after immersion of whole rats or decapitation into liquid N₂ (Veech *et al.*, 1972). The method of brain removal and freezing used in the present work minimizes post-mortem chemical changes.

The relationship between the changes in brain metabolite concentrations and the behavioural effects produced by morphine remains to be determined. It is worth noting, however, that the dysphoria associated with the 'hyperventilation syndrome' may be corrected rapidly by measures that elevate CO₂ concentration (Heyman, 1971). This suggests that part of the discomfort associated with narcotic withdrawal might be relieved by measures that increase CO₂ concentration.

Ether, phenobarbital and amobarbital anaesthesia in the mouse have been reported to cause increased brain glucose concentration and decreased pyruvate, lactate, α -oxoglutarate and malate concentrations (Lowry *et al.*, 1964; Mayman *et al.*, 1964; Gatfield *et al.*, 1966; Goldberg *et al.*, 1966). These changes in metabolite concentrations after anaesthesia are similar to the changes after administration of CO₂. The role of CO₂ was not evaluated in these studies. In a report on the effects of anaesthetics, CO₂ was held constant by artificial ventilation (Nilsson & Siesjö, 1970). Ether caused no change in lactate or pyruvate concentrations when compared with a control group given nitrous oxide anaesthesia. Phenobarbital and amobarbital, however, caused a decrease in lactate and pyruvate concentrations. These observations underline the importance of monitoring respiratory status in studies of general anaesthetics.

The cause of increased glucose concentrations with decreased concentrations of glycolytic and tricarboxylic acid-cycle intermediates observed after general anaesthetics has been attributed to depression of brain metabolic rate (Brunner *et al.*, 1971). A decrease in metabolic rate is unlikely to have caused the changes observed in the present study, as it has been shown that raising the CO₂ content of inspired air does not decrease brain O₂ consumption *in vivo* (Cohen *et al.*, 1964; Kety & Schmidt, 1948).

Takemori (1965) has reported increased glucose 6-phosphate dehydrogenase (EC 1.1.1.49) activity in brain after single injections of morphine. One consequence of [CO₂] elevation after acute morphine

injection is an increase in the cytoplasmic free [NADP⁺]/[NADPH] ratio. Whether the change in the [NADP⁺]/[NADPH] ratio is related to the increase in glucose 6-phosphate dehydrogenase activity is not known.

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