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ACUTE AND CHRONIC EFFECTS OF MORPHINE ON LIPOLYSIS IN RAT EPIDIDYMAL FAT PADS

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Abstract—The effects of morphine and adrenaline on lipolysis have been studied. *In vivo* studies show that both acute and chronic morphinization enhance lipase activity. *In vitro* addition of morphine produces a "dose-dependent" increase in the rate of fatty acid release, maximal activation being produced by 2.5 mM morphine. Chronically morphinized fat pad is insensitive to morphine added *in vitro*. However chronic morphinization does not completely abolish the sensitivity of the tissue to adrenaline. When normal fat pad is incubated in the presence of 2.5 mM morphine and submaximal concentrations of adrenaline (1–50 μM), there is an additive effect. Results from this study indicate that morphine may exert its effect via the same mechanism as adrenaline and suggest that the receptor on the plasma membrane has higher affinity for adrenaline than for morphine.

In a recent publication, it was reported that both acute and chronic morphinization enhanced the activities of two gluconeogenic enzymes in rat liver *in vivo* [1]. This effect may be explained on the basis that morphine, having a structure which is analogous to both adrenaline and hydrocortisone, may mimic the effects of the two hormones on the gluconeogenic enzymes. Since it is well established that adrenaline enhances lipolysis [2], it is therefore of interest to examine if morphine has a similar effect on adipose tissue.

In this paper, we report the results of a study of the *in vivo* effects of morphine on lipolysis in both acute and chronically treated animals, and the *in vitro* effects of morphine on the rate of fatty acid release by epididymal fat pads of both normal and chronically morphinized rats. Acute additive effect of adrenaline and morphine has also been investigated in normal rats.

MATERIALS AND METHODS

Animals. Male albino rats weighing 200–250 g were used throughout the experiment. They were allowed free access to food and water. Chronically morphinized animals received intraperitoneal (i.p.) injection of morphine according to the method of Gourley [3] for 17 days while control animals received the same volume of saline injection. Chronic morphinization had no effect on the well-being of the animal. Size of fat pads from chronically morphinized animals was not significantly different from those of normal control. Animals under acute treatment received only a single injection of morphine (30 mg/kg body wt) i.p. 1.5 hr before sacrifice.

Chemicals. All chemicals used were of analytical grade. Glycerol [1-^{14}C]trioleate was obtained from the Radiochemical Centre, Amersham. Tween 80, bovine serum albumin (fraction V), TEAE (Triethylaminoethyl)-cellulose, diethyldithiocarbamic acid (sodium salt) and adrenaline were obtained from Sigma Chemical Co. (St. Louis, Mo.).

Preparation of extract and determination of lipolytic activity. Rats were decapitated and their epididymal fat pads were immediately removed and washed in a solution of Krebs–Ringer bicarbonate buffer pH 7.4 which contained 3% "treated albumin" poor in fatty acid content [2]. Bovine serum albumin (fraction V) was pre-treated by the method of Goodman [4] to remove the endogenous fatty acid present in the preparation.

Tissue extracts were obtained by homogenizing the fat pads in 3 vol. of ice-cold 0.25 M sucrose solution in a glass chamber with a Teflon pestle and the supernatant was obtained by centrifugation at 16,000 *g* for 10 min [5]. Lipolytic activity in the supernatant was measured by the modification of the method of Zieve [6] in which the substrate was prepared by suspending 200 mg [1-^{14}C]triolein (sp. act. 0.15 μCi/μmole) in 10 ml of 100 mM Tris–HCl (pH 7.4) containing 5% bovine serum albumin, 20 mM $CaCl_2$ and 0.125% Tween 80 and sonicating for 1 min at room temperature. Reaction mixture contained 20 μl substrate in a final volume of 50 μl. The tubes were incubated for 50 min at 37° and stopped by addition of 1 ml of chloroform–methanol (5:1). The entire mixture was applied to a TEAE–cellulose column (0.5 × 3 cm). The column was washed with 20 ml of chloroform–methanol (5:1) and labelled fatty acid was then eluted with 3 ml of 0.2% acetic acid in methanol. The 3-ml eluate was collected directly into scintillation vial. The whole chromatographic procedure took 10–15 min.

For *in vitro* experiments, epididymal fat pads of size 0.35–0.50 g were incubated in Krebs–Ringer bicarbonate medium pH 7.4 containing 3% albumin (poor in fatty acids) for 1 hr in the presence of varying concentrations of morphine or adrenaline or both. As Rizack [5] has pointed out that preincubation of fat pads in a medium without glucose leads to inactiva-

Table 1. The *in vitro* effects of morphine on lipolysis

State of rats	No. of rats	Lipase activity (Mean ± S.E.M.)	Difference
Saline-treated (control)	5	0.355 ± 0.026	
			0.262 ± 0.09 ($P < 0.02$)
Morphine-treated for 1.5 hr (acute effect)	7	0.617 ± 0.073	
Saline-treated (control)	4	0.362 ± 0.017	
			0.204 ± 0.06 ($P < 0.02$)
Chronically morphinized	4	0.566 ± 0.062	

Lipase activity is expressed as μmoles fatty acid released from [1-^{14}C]triolein per g tissue per hr.

tion of lipase activity in the tissue extract, we adopted a more direct method for assaying lipolytic activity *in vitro* by measuring the amount of fatty acids released into the incubation medium by the method of Duncombe [7]. There is no direct evidence that endogenous reesterification of fatty acid in the fat pad is always negligible in the present experiment but from Vaughan and Steinberg's [8] study of hormone-sensitive lipase activity in adipose tissue, we can deduce that the rate of reesterification is very slow comparing to lipolysis. Vaughan and Steinberg assayed lipase activity by two different methods—determinations of glycerol produced and release of fatty acids. The ratio of fatty acid released to glycerol produced per g tissue per hr was approximately 3 (Lipase activity assayed by the 2 methods at pH 7.0 was 10.8 μmoles glycerol produced per g per hr and 31.8 μeq fatty acid released per g per hr respectively).

RESULTS

Acute and chronic effects of morphine administration on lipase activity in vivo. Acute treatment of rats with morphine 1 hr prior to sacrifice has no effect on lipase activity (data not presented here) but as the period of exposure to the drug is prolonged to 1.5 hr, there is a significant increase of lipase activity (74% elevation) as shown in Table 1. The lipase activity from chronically treated animals is significantly higher than that of normal control but the extent of increase after chronic treatment is only 56 per cent.

Effects of varying morphine concentration on fatty acid release by isolated epididymal fat pads of normal and of chronically morphinized rats. Our results show that morphine enhances the rate of fatty acid release from epididymal fat pads, maximal rate being attained with a morphine concentration of 2.5 mM (Table 2). On the other hand, fat pads from chronically morphinized animals have lost their sensitivity to morphine added *in vitro*. Though the rate of fatty acid release by epididymal fat pads from chronically morphinized rats is significantly greater than that of normal (difference being 0.92 ± 0.09, $P < 0.001$), there is no further increase in the release of fatty acids upon *in vitro* addition of morphine.

In vitro *effects of adrenaline on fatty acid release by epididymal fat pads of normal and of chronically morphinized rats.* Our results show that in normal rats, the saturating dosage of adrenaline is 100 μM. Further increase of the albumin in the assay to 6% does not enhance fatty acid release (Table 5). However, with epididymal fat pads from chronically treated animals, the stimulatory effect of adrenaline still persists but the maximal activity achieved has dropped by 53 per cent (Table 3). Thus chronic morphinization has not completely abolished the sensitivity of the tissue to adrenaline but the extent of increase of fatty acid release is lower than that demonstrated in normal rats.

Combined effects of morphine and adrenaline on fatty acid release by epididymal fat pads of normal rats. Morphine does not modify the effect of adrenaline

Table 2. The *in vitro* effects of morphine on fatty acid release by epididymal fat pads of normal and of chronically morphinized rats

	Normal Rats		Chronically morphinized rats	
Concn. of Morphine (mM)	No. of rats	Rate of fatty acid release (Mean ± S.E.M.)	No. of rats	Rate of fatty acid release (Mean ± S.E.M.)
0	5	0.95 ± 0.04	6	1.87 ± 0.08
0.50	5	1.46 ± 0.31	—	—
1.25	5	1.62 ± 0.10	6	1.98 ± 0.20
2.50	5	3.05 ± 0.13	6	1.93 ± 0.14
3.75	5	1.77 ± 0.12	—	—
5.00	5	1.47 ± 0.11	6	2.16 ± 0.13

Fat pads of size 0.35–0.50 g were incubated at pH 7.4 and 37° for 1 hr in oxygenated Krebs–Ringer bicarbonate medium (2 ml) containing 3% albumin ± morphine at varying concentrations. Rate of fatty acid release is expressed as μmoles of fatty acids released per g tissue per hr.

Table 3. The *in vitro* effects of adrenaline on fatty acid release by epididymal fat pads of normal and of chronically morphinized rats

Concn. of Adrenaline (μM)	Normal Rats		Chronically morphinized rats	
	No. of rats	Rate of fatty acid release (Mean ± S.E.M.)	No. of rats	Rate of fatty acid release (Mean ± S.E.M.)
0	12	0.91 ± 0.05	6	1.53 ± 0.14
1	4	2.16 ± 0.12	6	2.19 ± 0.23
5	7	3.22 ± 0.54	6	3.25 ± 0.30
10	9	5.06 ± 0.53	6	3.00 ± 0.13
50	6	6.10 ± 1.06	6	3.27 ± 0.33
100	9	6.97 ± 0.60	—	—
200	4	6.43 ± 1.38	—	—

Fat pads of size 0.35–0.50 g were incubated at pH 7.4 and 37° for 1 hr in oxygenated Krebs–Ringer bicarbonate medium (2 ml) containing 3% albumin ± adrenaline at varying concentrations. Rate of fatty acid release is expressed as μmoles of fatty acid released per g tissue per hr.

in stimulating fatty acid release from adipose tissue. Since 2.5 mM morphine has been shown to be the concentration that produced maximal effect (Table 2), it is chosen as the concentration for additive studies. At submaximal concentrations of adrenaline (from 1 μM to 50 μM), addition of 2.5 mM morphine produces additive results, but when solution of this concentration of morphine is added to a 100 μM adrenaline solution (saturating dosage), the effects are not additive (Table 4). Increasing albumin concentration in the incubation medium to 6% does not alter the result of this investigation (Table 5).

DISCUSSION

It is obvious that a single dose of morphine does increase the lipolytic activity in rat adipose tissue (Table 1). Though injections of morphine generally promote the release of adrenaline from the adrenal medulla [9, 10], rats are much less sensitive than other animals. Outschoorn [11] reported that after only one injection of morphine, there was no appreciable change of adrenaline content in the adrenal gland within 20 hr while a significant depletion to 43 per cent of the original value was achieved with four

Table 4. Effect of *in vitro* addition of morphine and adrenaline on fatty acid release by epididymal fat pads of normal rats

No. of rats	Concn. of Adrenaline (μM)	Control (Mean ± S.E.M.)	+2.5 mM Morphine (Mean ± S.E.M.)	Difference (Mean ± S.E.M.)
5	0	0.95 ± 0.3	2.98 ± 0.12	2.03 ± 0.14 ($P < 0.001$)
4	1	2.06 ± 0.18	3.68 ± 0.14	1.62 ± 0.20 ($P < 0.01$)
6	10	4.94 ± 0.40	6.49 ± 0.62	1.55 ± 0.15 ($P < 0.001$)
8	100	6.45 ± 0.58	6.52 ± 0.47	0.07 ± 0.30 (N.S.)

Fat pads of size 0.35–0.50 g were incubated at pH 7.4 and 37° for 1 hr in oxygenated Krebs-Ringer bicarbonate medium (2 ml) containing 3% albumin ± morphine final concentration 2.5 mM and in which the adrenaline concentration was varied. In each experiment one fat pad served as a control for the other. Mean ± S.E.M. are expressed as μmoles fatty acid released per g tissue per hr.

Table 5. Effects of different concentrations of albumin on fatty acid release by epididymal fat pads of normal rats

No. of rats	Drugs added	Rate of fatty acid release (Mean ± S.E.M.)		Difference (Mean ± S.E.M.)
		3% Albumin	6% Albumin	
4	+ 100 μM Adrenaline	6.76 ± 0.78	6.31 ± 0.90	− 0.45 ± 0.41 (N.S.)
4	+ 100 μM Adrenaline + 2.5 mM Morphine	6.42 ± 0.90	6.19 ± 1.01	− 0.23 ± 0.67 (N.S.)

Fat pads of size 0.35–0.50 g were incubated at pH 7.4 and 37° for 1 hr in oxygenated Krebs–Ringer bicarbonate medium (2 ml) containing adrenaline final concentration 100 μM ± morphine final concentration 2.5 mM and in which the albumin concentration was varied. In each experiment, one fat pad served as a control for the other. Mean ± S.E.M. are expressed as μmoles fatty acid released per g per hr.

injections of the drug at hourly intervals. This was later confirmed by Crawford [12] who found that four injections of morphine at 1-hr intervals evoked a definite rise in excretion of adrenaline in the 24-hr urine sample although a single injection of the drug did not have any effect. Since the present *in vivo* study is done with a single injection of morphine, it is unlikely that the activation of lipase activity is due to an increased release of adrenaline from the adrenal medulla. This dose of morphine (30 mg/kg body wt) can usually produce analgesia in 30 min (tested by hot-plate technique). In the present study, it is found that there is a lag period of 1.5 hr before an effect on lipase activity can be demonstrated. Thus it is not certain whether this increase in lipase activity is a consequence of the direct action of morphine or is secondary to some other primary action in the whole animal. It is therefore desirable to investigate the effect of morphine on isolated fat pads.

It can be seen from Table 2 that morphine does increase the amount of fatty acid release from isolated epididymal fat pads. This suggests that morphine has a direct effect on adipose tissue. The stimulatory effect of morphine *in vitro* is "dose-dependent", with a maximum being attained at 2.5 mM. Upon further increase in morphine concentration the rate reverts to normal. However this stimulatory effect of morphine *in vitro* is completely lost after chronic treatment. It appears that cells of adipose tissue have become adapted during chronic morphinization so that they no longer respond to morphine added *in vitro*. Cellular adaptation to morphine in rats had been reported by many workers on various aspects [13–18]. Takemori [13] reported that morphine at a concentration of 1×10^{-3}M inhibited the K^+-stimulated oxygen uptake of cerebral cortical slices from normal rats but failed to alter the stimulatory respiratory rate of slices from chronically treated animals. Hano *et al.* [14, 15] also showed cellular adaptation to morphine in rats made tolerant to 120 mg/kg body wt. Walsh *et al.* [16] also reported that the stimulatory effect of insulin on glucose-uptake by diaphragm of normal rats was lost after chronic morphinization. Besides, sensitivity of glucose-uptake by diaphragm to extracellular magnesium and potassium has also been abolished after chronic morphinization [17, 18]. The present finding is in accordance with the idea of cellular adaptation during chronic treatment. Though the rate of fatty acid release by epididymal fat pads from chronically morphinized rats is significantly greater than that of normal, it is not certain whether this is a result of an increase in amount of enzyme synthesized or activation of preformed enzyme molecules. Inhibitors of protein synthesis have not been used in the present study because Loh *et al.* [19] have shown that concomitant administration of protein synthesis inhibitors with morphine prevent the development of tolerance and physical dependence.

It might therefore be expected that the chronically morphinized fat pads would be insensitive to the stimulatory effect of adrenaline. Our results show that this is not the case: the chronically morphinized fat pads still possess sensitivity to adrenaline, but the effect, compared with that of normal tissue, is greatly reduced and being saturated at a lower concentration of adrenaline (Table 3). Thus we were confronted with the problem whether adrenaline and morphine act differently. The problem may be resolved by testing the additivity of the effects of the two compounds. Our experimental results (Table 4) provide suggestive evidence that the two compounds may have a similar site of action, because at saturated concentration of adrenaline (100 μM), addition of morphine cannot produce any further increase in fatty acid release. This claim is valid only if the acceptor sites on the albumin are not saturated. Goodman [20] in studying the binding properties of serum albumin has reported that there are seven strong binding sites and more than twenty weaker sites for long chain fatty acids. We further confirm that the albumin present in the incubating medium is not rate-limiting by our finding that on increasing the concentration of albumin from 3 to 6%, no further increase in fatty acid release can be demonstrated (Table 5).

Since a much lower concentration of adrenaline can stimulate fatty acid release to the same extent as that by morphine (fatty acids released in the presence of 5 μM adrenaline is being equivalent to that in the presence of 2.5 mM morphine), it is speculated that the receptor has a much higher affinity for adrenaline than for morphine. Dunnick and Marinette [21] have shown that the two hydroxy groups of the benzene ring of adrenaline are important in the binding of the hormone to the plasma membrane thereby augmenting the cyclic AMP level. As morphine also possesses two hydroxy groups and a benzene ring, it may bind to the same site on the membrane and may also affect the cyclic AMP level. Morphine has been shown to potentiate the cyclic AMP level in the brain by many workers. Costa *et al.* [22] reported an increase in striated cyclic AMP level by acute morphine administration. Chou *et al.* [23] found that morphine significantly increased the activity of adenylate cyclase in mouse cerebral cortex. A recent study by Puri *et al.* [24] has shown that i.p. administration of morphine (30 mg/kg 1 hr before sacrifice) caused a significant increase in the striatal adenylate cyclase activity. The same authors found that morphine did inhibit phosphodiesterase both *in vitro* and *in vivo* when a high substrate concentration (3.3×10^{-3}M cyclic AMP) was used. It is therefore speculated that morphine may promote an increase of cyclic AMP content in the adipose tissue. However the present study does not rule out other possible mechanisms whereby morphine can exert its effects.

Since chronic morphinization may have induced changes in the membrane structure [18], the receptor's affinity of both adrenaline and morphine would be altered. As a consequence of chronic morphinization, the receptors may have lower affinity than those from normal tissue. Hence only adrenaline can bind to the receptor on the membrane of adipose tissue from chronically morphinized rats while morphine is without effect. This may offer an explanation for the differential effect of chronic morphinization on the sensitivity to adrenaline and morphine when given *in vitro*.

REFERENCES

1. S. C. Wong, D. Yeung and K. S. Au, *Biochem. Pharmac.* **23,** 829 (1974).

2. R. W. Butcher, R. J. Ho, H. C. Meng and E. W. Sutherland, *J. biol. Chem.* **240,** 4515 (1965).
3. D. R. H. Gourley, *Biochem. Pharmac.* **23,** 489 (1974).
4. D. S. Goodman, *Science, N.Y.* **125,** 1297 (1957).
5. M. A. Rizack, *J. biol. Chem.* **236,** 657 (1961).
6. F. J. Zieve, *Analyt. Biochem.* **51,** 436 (1973).
7. W. G. Duncombe, *Biochem. J.* **88,** 7 (1963).
8. M. Vaughan, J. E. Berger and D. Steinberg, *J. biol. Chem.* **239,** 401 (1964).
9. J. A. Richardson, E. F. Woods and A. K. Richardson, *J. Pharmac. exp. Ther.* **122,** 64A (1958).
10. E. W. Maynert and G. I. Klingman, *J. Pharmac. exp. Ther.* **135,** 285 (1962).
11. A. S. Outschoorn, *Br. J. Pharmac.* **7,** 605 (1952).
12. T. B. B. Crawford and W. Law, *Br. J. Pharmac.* **13,** 35 (1958).
13. A. E. Takemori, *J. Pharmac. exp. Ther.* **135,** 89 (1962).
14. K. Hano, H. Kaneto and T. Kakunaya, *Folia Pharmac. Japan* **58,** 114 (1962).
15. K. Hano, H. Kaneto and T. Kakunaya, *Folia Pharmac. Japan* **59,** 71 (1963).
16. E. O'F. Walsh, C. H. Lee Peng and S. C. Wong, *Biochem. Pharmac.* **18,** 1529 (1969).
17. M. W. Poon, E. O'F. Walsh and M. L. Ng, *Biochem. Pharmac.* **17,** 1575 (1968).
18. S. C. Wong and E. O'F. Walsh, *Biochem. Pharmac.* **18,** 1979 (1969).
19. H. H. Loh, F. H. Shen and E. L. Way, *Biochem. Pharmac.* **18,** 2711 (1969).
20. D. S. Goodman, *J. Am. chem. Soc.* **80,** 3892 (1958).
21. J. K. Dunnick and G. V. Marinette, *Biochim. biophys. Acta* **249,** 122 (1971).
22. E. Costa, A. Carenzi, A. Guidotti and A. Revuelta, *Frontiers in Catecholamine Research*, p. 1003. Pergamon Press, London (1973).
23. W. S. Chou, A. K. S. Ho and H. H. Loh, *Proc. West. pharmac. Soc.* **14,** 42 (1971).
24. S. K. Puri, J. Cochin and L. Volicer, *Life Sci.* **16**(5), 759 (1975).