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EFFECTS OF MORPHINE ON THE HORMONAL CONTROL OF METABOLISM—III

EFFECTS *IN VITRO* OF MORPHINE AND ADRENALINE ON UTILIZATION OF GLUCOSE BY MUSCLE OF NORMAL AND CHRONICALLY MORPHINIZED RATS

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Abstract—The effects of morphine and adrenaline, acting singly and together, on glucose uptake, glycogen metabolism, glycolysis, and respiration of excised diaphragms of normal and chronically morphinized rats have been studied *in vitro*.

Differences between normal and chronically morphinized tissue in response both to morphine and to adrenaline are observed, but only in the effects on glucose uptake.

Under experimental conditions in which adrenaline retards glucose uptake by normal tissue, chronically morphinized tissue is insensitive to adrenaline, but sensitivity is restored by addition of morphine. This effect of morphine is similar to that of hydrocortisone which also restores adrenaline-sensitivity to chronically morphinized tissue. The direct effect of morphine on chronically morphinized tissue is similar to that of adrenaline on normal tissue.

The experimental evidence indicates that as a result of morphine-induced changes in skeletal muscle, the tissue responds abnormally to adrenal hormones, but certain effects of both types of adrenal hormone on normal tissue are simulated by morphine when acting on chronically morphinized tissue.

IN PREVIOUS papers,[1–3] the results have been reported of a comparative study of the acute effects of morphine and hydrocortisone, acting separately and in the presence of each other, and of hydrocortisone and adrenaline, acting separately and in the presence of each other, on glucose uptake, glycogen metabolism, lactate accumulation, and oxygen consumption by isolated diaphragm of both normal and chronically morphinized rats. We now report the results of an analogous study of the effects of morphine and adrenaline in relation to each other.

This work completes a preliminary survey of the chronic and acute effects of morphine in relation to the effects on carbohydrate metabolism of adrenal hormones at a target site. The results add further support to the thesis[4] that repeated dosage with morphine induces changes whereby the drug assumes the role of a pseudohormone participating in the adrenal complex of factors involved in the control of metabolism.

MATERIALS AND METHODS

The animals and materials used and the experimental procedures were as previously described.[1, 2] The additives to the incubation media were morphine and/or adrenaline, the final concentration of each additive being $3{\cdot}85 \times 10^{-4}$ M. Some attempts were made in the present series of experiments to use the glucose oxidase method for

estimations of glucose but adrenaline interfered and the Nelson method[5] was therefore used in all experiments here reported.

EXPERIMENTAL AND RESULTS

Effects of morphine and adrenaline on glucose-uptake by isolated diaphragm of normal and of chronically morphinized rats

As shown in the "paired technique" experiments (Table 1), the stimulant effect of morphine on the rate of glucose uptake from oxygenated, phosphate-buffered medium by normal diaphragm is not antagonized by the presence of adrenaline (see experiments 1 and 4) neither is the depressant effect of adrenaline affected by the presence of morphine (see experiments 2 and 5). In the chronically morphinized tissue on the other hand, the characteristically depressant effect of morphine is antagonized by adrenaline which alone has no effect on the rate of glucose uptake. In the presence of morphine, however, adrenaline depresses glucose uptake in the chronically morphinized diaphragm to an extent comparable with its effect when acting alone on normal tissue.

TABLE 1. EFFECTS OF MORPHINE AND ADRENALINE ON UPTAKE OF GLUCOSE BY ISOLATED DIAPHRAGM OF NORMAL AND OF CHRONICALLY MORPHINIZED RATS

State of rat	No. of rats	Control	Experiment	Difference
			+Morphine	
N	8	198 ± 32	252 ± 20	+ 54 ± 18 ($P < 0.02$)
C m	8	238 ± 10	160 ± 22	− 78 ± 16 ($P < 0.002$)
			+Adrenaline	
N	5	161 ± 14	41 ± 12	−120 ± 15 ($P < 0.001$)
C m	6	171 ± 50	175 ± 58	+ 4 ± 50
			+Morphine +Adrenaline	
N	6	321 ± 32	141 ± 22	−180 ± 51 ($P < 0.02$)
C m	6	373 ± 38	257 ± 47	−116 ± 25 ($P < 0.01$)
		+Adrenaline	+Morphine +Adrenaline	
N	11	148 ± 17	233 ± 31	+ 85 ± 25 ($P < 0.01$)
C m	12	304 ± 21	274 ± 27	− 30 ± 31
		+Morphine	+Morphine +Adrenaline	
N	8	314 ± 24	180 ± 31	−134 ± 32 ($P < 0.01$)
C m	8	319 ± 34	172 ± 18	−147 ± 35 ($P < 0.01$)

Hemi-diaphragms were incubated at pH 7·4 and 37° for 1 hr in oxygenated Krebs-Ringer-phosphate (2·0 ml) containing glucose (0·15%) ± added hormone and/or drug (each in final concentration, $3{\cdot}85 \times 10^{-4}$ M). In each experiment, half the excised diaphragm served as a control for the other half. Mean rates ± S.E. mean are expressed as decrease in glucose content of the medium, mg/100 g wet tissue per hr.

N—normal; *C m*—chronically morphinized.

Effects of morphine and adrenaline on glycogen metabolism in isolated diaphragm of normal and of chronically morphinized rats

As shown in the "paired technique" experiments (Table 2), morphine does not interfere with the effects of adrenaline on glycogenolysis in either normal or chronically morphinzed diaphragm.

TABLE 2. EFFECTS OF MORPHINE AND ADRENALINE ON GLYCOGEN METABOLISM IN ISOLATED DIAPHRAGM OF NORMAL AND OF CHRONICALLY MORPHINIZED RATS

State of rat	No. of rats	Control	Experiment	Difference
			+Morphine	
N	8	+45 ± 3	+50 ± 9	+ 5 ± 22
C m	8	+55 ± 8	+50 ± 8	− 5 ± 8
			+Adrenaline	
N	5	+36 ± 6	− 8 ± 3	− 44 ± 7 ($P < 0.01$)
C m	7	+34 ± 10	−53 ± 13	− 87 ± 19 ($P < 0.01$)
			+Morphine +Adrenaline	
N	6	+62 ± 12	+21 ± 4	− 41 ± 14 ($P < 0.05$)
C m	6	+70 ± 16	−43 ± 20	−113 ± 8 ($P \ll 0.001$)
		+Adrenaline	+Morphine +Adrenaline	
N	8	+29 ± 5	+25 ± 5	− 4 ± 8
C m	8	−47 ± 7	−41 ± 7	+ 6 ± 7
		+Morphine	+Morphine +Adrenaline	
N	8	+69 ± 12	+14 ± 10	− 55 ± 10 ($P < 0.001$)
C m	8	+54 ± 12	−23 ± 8	− 77 ± 10 ($P \ll 0.001$)

In each experiment, the freshly excised diaphragm was trisected, the middle, vertebral portion used for estimation of initial glycogen content and the two similar, lateral portions, one serving as control for the other, were incubated separately at pH 7·4 and 37° for 2 hr in oxygenated Krebs-Ringer-phosphate (2·0 ml) containing glucose (0·15%) ± added hormone and/or drug (each in final concentration, $3{\cdot}85 \times 10^{-4}$ M). Mean rates of change of glycogen content ± S.E. mean are expressed as increase (+) or decrease (−) of glucose equivalent, mg/100 wet tissue per hr.

N—normal; *C m*—chronically morphinized.

Effects of morphine and adrenaline on lactate accumulation in isolated diaphragm of normal and of chronically morphinized rats

Neither morphine nor adrenaline alone significantly affect the rate of lactate accumulation in oxygenated, phosphate-buffered medium by isolated diaphragm of either normal or chronically morphinized rats. A small increase in the rate is observed in some groups of experiments (Table 3) when both morphine and adrenaline are present, but no consistent difference in behaviour between normal and chronically morphinized tissue is revealed by comparison of effects on lactate accumulation.

Effects of morphine and adrenaline on uptake of oxygen by isolated diaphragm of normal and of chronically morphinized rats

As shown in the "paired technique" experiments (Table 4), morphine does not modify the effect of adrenaline in stimulating respiration of diaphragm in oxygenated, phosphate-buffered medium and no appreciable difference between normal and chronically morphinized tissue is revealed by comparison of the effects of the drug and hormone on the rates of oxygen uptake.

Comparison of basal metabolic rates in isolated diaphragm of normal and of chronically morphinized rats

In Table 5 the mean values for rates of glucose uptake, net glycogen synthesis, lactate accumulation, and oxygen consumption of diaphragm from normal rats is compared with that from chronically morphinized rats. The means have been calculated from values obtained with the control hemi-diaphragms used in the experiments reported in this and the preceding papers[1, 3] and involving about 140 rats,

TABLE 3. EFFECTS OF MORPHINE AND ADRENALINE ON LACTATE ACCUMULATION IN ISOLATED DIAPHRAGM OF NORMAL AND OF CHRONICALLY MORPHINIZED RATS

State of rat	No. of rats	Control	Experiment	Difference
			+Morphine	
N	8	63 ± 6	63 ± 2	0 ± 6
C m	8	83 ± 7	82 ± 6	− 1 ± 8
			+Adrenaline	
N	6	77 ± 5	80 ± 4	+ 3 ± 5
C m	8	79 ± 4	86 ± 4	+ 7 ± 4
			+Morphine +Adrenaline	
N	6	78 ± 5	99 ± 8	+21 ± 5 ($P < 0.01$)
C m	6	84 ± 5	91 ± 6	+ 7 ± 6
		+Adrenaline	+Morphine +Adrenaline	
N	8	83 ± 2	86 ± 2	+ 3 ± 4
C m	8	100 ± 5	100 ± 5	0 ± 5
		+Morphine	+Morphine +Adrenaline	
N	8	75 ± 2	84 ± 1	+ 9 ± 1·7 ($P < 0.002$)
C m	8	79 ± 2	91 ± 3	+12 ± 3 ($P < 0.01$)

Hemi-diaphragms were incubated at pH 7·4 and 37° for 1 hr in oxygenated Krebs-Ringer-phosphate (2·0 ml) containing glucose (0·15%) ± added hormone and/or drug (each in final concentration, $3{\cdot}85 \times 10^{-4}$ M). In each experiment, half the excised diaphragm served as control for the other half. Mean rates ± S.E. mean are expressed as increase in lactic acid content of the medium, mg/100 g wet tissue per hr.

N—normal; *C m*—chronically morphinized.

half of which were chronically morphinized. Apart from the suggestion that net glycogen synthesis proceeds at a slightly higher rate in the chronically morphinized group, though the difference (+ 0·39 ± 0·33 μmole/g wet tissue/hr) is not significant, there is virtually no difference between the two groups and it is confirmed that the basal rates of glucose-uptake, glycolysis, and respiration are not changed as a result of, chronic morphinization.

DISCUSSION

The acute, effects *in vitro* of morphine, hydrocortisone, and adrenaline on the rate of glucose-uptake by isolated diaphragm from chronically morphinized rats are in each case different from the effects on diaphragm from normal rats. In summary of

this and previous work,[1-3] morphine accelerates while both hydrocortisone and adrenaline retard glucose uptake by normal diaphragm, whereas morphine retards, hydrocortisone accelerates, and adrenaline is without effect on the rate of uptake by chronically morphinized diaphragm.

TABLE 4. EFFECTS OF MORPHINE AND ADRENALINE ON UPTAKE OF OXYGEN BY ISOLATED DIAPHRAGM OF NORMAL AND OF CHRONICALLY MORPHINIZED RATS

State of rat	No. of rats	Control	Experiment	Difference
			+Morphine	
N	8	1195 ± 41	1193 ± 69	− 2 ± 91
C m	8	1194 ± 59	1213 ± 39	+19 ± 77
			+Adrenaline	
N	10	919 ± 30	1015 ± 26	+ 96 ± 22 ($P < 0.002$)
C m	10	1089 ± 35	1205 ± 50	+113 ± 41 ($P < 0.05$)
			+Morphine +Adrenaline	
N	6	953 ± 151	1128 ± 89	+175 ± 39 ($P < 0.01$)
C m	6	1047 ± 37	1218 ± 84	+171 ± 62 ($P < 0.05$)
		+Adrenaline	+Morphine +Adrenaline	
N	8	1243 ± 39	1348 ± 54	+105 ± 54
C m	8	1247 ± 69	1302 ± 59	+ 55 ± 56
		+Morphine	+Morphine +Adrenaline	
N	8	1121 ± 54	1227 ± 42	+106 ± 40 ($P < 0.05$)
C m	8	1316 ± 53	1445 ± 67	+129 ± 41 ($P < 0.02$)

Hemi-diaphragms were incubated with shaking in oxygenated Krebs-Ringer-phosphate (2·0 ml) containing glucose (0·15%) ± added hormone and/or drug (each in final concentration, $3{\cdot}85 \times 10^{-4}$ M) at pH 7·4 and 37° for 1 hr. O_2- uptakes were measured by the Warburg direct method with O_2 as gas phase, the centre wells containing 30% KOH (0·2 ml). In each experiment, half the excised diaphragm served as control for the other half. Mean rates ± S.E. mean are expressed as μl O_2/g wet tissue per hr.

N—normal; *C m*—chronically morphinized.

TABLE 5. COMPARISON OF BASAL METABOLIC RATES IN ISOLATED DIAPHRAGM OF NORMAL AND OF CHRONICALLY MORPHINIZED RATS

State of rat	Glucose uptake	Net glycogen synthesis	Lactate accumulation	Oxygen consumption
N	(67) 11·89 ± 0·72	(62) 1·67 ± 0·21	(62) 9·89 ± 0·38	(60) 44·54 ± 1·34
C m	(65) 11·83 ± 0·83	(63) 2·06 ± 0·27	(68) 10·33 ± 0·41	(62) 47·44 ± 1·06

All values are expressed as a mean ± S.E. mean in μmoles/g wet tissue per hr, glucose equivalent in the case of glycogen, with the number of rats shown in parenthesis. In each experiment hemi-diaphragms were incubated at pH 7·4 and 37° with shaking in oxygenated Krebs-Ringer-phosphate (2·0 ml) containing glucose (0·15%).

N—normal; *C m*—chronically morphinized

Thus, with reference to effects on glucose-uptake, the tissue from a chronically morphinized animal responds abnormally to both types of adrenal hormone and the acute effect of morphine on the chronically morphinized tissue simulates that of either

hydrocortisone or adrenaline on normal tissue. Considered in isolation, this would suggest that repeated exposure of the tissue to morphine has induced a change of an adaptive nature whereby the normal function of the adrenal hormones has been taken over by the drug, which thus assumes the role of a pseudohormone in which both adrenaline-like and steroid-like properties are combined. As previously pointed out,[1] the morphine molecule embodies both an adrenaline-like and a sterioid-like structure.

On the other hand, morphine does not appreciably interfere with the intrinsic controlling mechanisms of cellular metabolism or with the effects of adrenal hormones on the intracellular processes concerned with glycolysis and respiration. It is not therefore suggested that the function of adrenal hormones is entirely usurped by the drug in the chronically morphinized animal, but its presence would appear to be necessary to preserve a balance of hormonal effects comparable with that achieved in the normal animal by the natural hormones alone. This could well account for the addicting properties of morphine.

The picture would be relatively clear if one could say simply that certain functions of the adrenal hormones, those concerned with activity at the membrane, had been taken over by morphine, while those concerned with intracellular mechanisms continued to operate normally. A variety of factors obscure the picture, however. Apart from the fact that morphine possesses potent pharmacological properties, acting directly on the nervous system and also influencing endocrine activity, there is the further complication that the effects of the drug and of the hormones on the glucose transport system are not entirely independent of one another, the acute effect of any one being subject to modification by one or more of the others, and differences in the nature of such modification become manifest when effects on normal tissue are compared with those on chronically morphinized tissue. For example, though morphine and adrenaline have opposing effects on normal diaphragm, neither appears to interfere directly with the action of the other, since adrenaline depresses glucose uptake whether morphine is present or not and morphine accelerates glucose uptake whether adrenaline is present or not. With chronically morphinized tissue on the other hand, sensitivity to adrenaline is dependent on the presence either of morphine or, as previously reported,[3] of hydrocortisone.

In view of the steroid-like effects of morphine on chronically morphinized tissue it is of interest to note that its effect on normal tissue is similar to that of the steroid on chronically morphinized tissue, and it is also similar to that of the steroid on normal tissue in the presence of adrenaline.

The effect of morphine on glucose-uptake by normal diaphragm may be a hormone-simulating (insulin-like) effect or, alternatively, it could be regarded as a toxic effect since a variety of adverse conditions,[6] such as lack of oxygen, exposure to various metabolic inhibitors or uncoupling agents, or drastic modifications in the ionic composition of the medium[7] such as omission of potassium or calcium or elevation of the magnesium content to abnormally high levels, all result in an increased rate of glucose uptake.

The latter interpretation would imply that only in its effect on the tissue from a chronically morphinized animal does morphine have a true hormone-like effect and that the reversed effect of hydrocortisone on such tissue is possibly a toxic effect analogous to that of morphine on normal tissue. We do not favour this latter interpretation, however, since morphine, in even higher concentration than that used in our

experiments, appears not to interfere with the general metabolism or to disturb the normal rate of respiration of muscle tissue preparations. On the other hand, we hesitate to describe the effect of morphine on normal tissue as an insulin-like effect. Gemmill and Hamman[8] found that the major part of the extra glucose taken up by rat diaphragm under the influence of insulin could be accounted for as glycogen and others[9–11] have confirmed that insulin stimulates glycogen synthesis, but we cannot claim to have demonstrated such an effect with morphine, though the possibility remains, and this we propose to investigate, that morphine accelerates glycogenesis and glycogenolysis to a similar extent. This seems unlikely, however, and such an effect, resulting in an increased rate of carbohydrate metabolism, would presumably have been revealed by an increase in lactate or in oxygen consumption.

Although with a physiologically normal extracellular glucose concentration the rate of glucose uptake is generally regarded as the rate-limiting step in carbohydrate metabolism of muscle, it would appear that in the resting state, as in experiments *in vitro*, an increased rate of glucose uptake is not necessarily followed by an increase in the rate of carbohydrate metabolism. For example, an increased rate of uptake caused by raising the glucose concentration of the incubation medium is not accompanied by an increased rate of glycogen synthesis.[12] The effect of morphine is similar in this respect and it would appear that the influence of the drug is confined entirely to the membrane.

In seeking an explanation for the different effect on chronically morphinized tissue it is reasonable to assume that it is in the membrane that morphine-induced change has occurred and, as revealed by changes in sensitivity and response to adrenal hormones, that such change involves modification of the hormone-sensitive apparatus for controlling the rate of glucose-uptake. Until we know precisely how the hormones exert their influence on the membrane any attempts to explain the effects of morphine must necessarily be speculative. Further investigation is in progress.

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REFERENCES

1. C. H. Lee Peng and E. O'F. Walsh, *Biochem. Pharmac.* **12**, 921 (1963).
2. E. O'F. Walsh, C. H. Lee Peng and M. L. Ng, *Nature, Lond.* **204**, 698 (1964).
3. M. L. Ng and E. O'F. Walsh, *Biochem. Pharmac.* **14**, 1003 (1965).
4. E. O'F. Walsh, *Med. News, N.Y.* No. 136, 13 (1965).
5. N. Nelson, *J. biol. Chem.* **153**, 375 (1944).
6. P. J. Randle, *Membrane Transport and Metabolism* (Eds. A. Kleinzeller and A. Kotyk), p. 434. Academic Press, London (1961).
7. M. W. Poon and E. O'F. Walsh, to be published.
8. C. L. Gemmill and L. Hamman, Jr., *Bull. Johns Hopkins Hosp.* **68**, 50 (1941).
9. N. Haugaard and W. C. Stadie, *J. biol. Chem.* **189**, 59 (1951).
10. W. N. Shaw and W. C. Stadie, *J. biol. Chem*, **227**, 115 (1957).
11. W. N. Shaw and W. C. Stadie, *J. biol. Chem.* **234**, 2491 (1959).
12. J. Larner, C. Villar-Palasi, and D. J. Richman, *Ann. N.Y. Acad. Sci.* **82**, 845 (1959).