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EFFECTS OF MORPHINE ON THE HORMONAL CONTROL OF METABOLISM—II.

IN VITRO EFFECTS OF ADRENALINE AND HYDROCORTISONE ON UTILIZATION OF GLUCOSE BY MUSCLE OF NORMAL AND CHRONICALLY MORPHINISED RATS

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

Under experimental conditions in which both adrenaline and hydrocortisone retard glucose-uptake by normal tissue, adrenaline is without effect and hydrocortisone accelerates glucose-uptake by chronically morphinised tissue. In the presence of adrenaline, hydrocortisone accelerates glucose-uptake by normal but not by chronically morphinised tissue. In the presence of hydrocortisone, adrenaline retards glucose-uptake by chronically morphinised tissue.

Such marked differences between normal and chronically morphinised tissue are not manifest in the effects of either hormone on intracellular carbohydrate metabolism and it is concluded that the cell membrane is the site of morphine-induced changes.

Previous work has established that morphine has a direct and hormone-simulating effect on muscle metabolism[1] and it has been suggested that the addicting properties of the drug may be associated with the embodiment in the morphine molecule of structures analogous both to steroid hormones and to adrenaline.[2] The discovery[2] that morphine and hydrocortisone each antagonise or modify the effects of the other on certain metabolic processes, and that *in vitro* responses to both hormone and drug are different in tissue from chronically morphinised animals, supports this view in so far as analogy between the drug and a steroid hormone is concerned.

We now report the results of a study of the effects on glucose-uptake and metabolism, in diaphragm of both normal and chronically morphinised rats, of hydrocortisone and adrenaline, acting singly and together as in the previous experiments with hydrocortisone and morphine.

It is demonstrated that certain effects of both these hormones are modified, and in some cases actually reversed, in tissue from chronically morphinised animals.

MATERIALS AND METHODS

THE animals and materials used and the experimental procedures were as previously described[2] with the following exception. The additives to the incubation media were adrenaline and/or hydrocortisone instead of morphine and/or hydrocortisone and the final concentrations of each additive were $3{\cdot}85 \times 10^{-4}$ M instead of $7{\cdot}7 \times 10^{-4}$ M, the higher concentration being impractical in the case of adrenaline. Preliminary experiments indicated that maximal effects of adrenaline were obtained with the concentration used in the present work. This is a higher concentration than is normally

used in experiments with adrenaline but, in these preliminary investigations, we wish to compare the effects and to study influence upon one another of the hormones and the drug in equimolecular concentrations.

RESULTS

Effects of adrenaline and hydrocortisone on glucose-uptake by isolated diaphragm of normal and chronically morphinised rats

As shown in the 'paired technique' experiments (Table 1), adrenaline and hydrocortisone when acting separately in the concentrations here used ($3{\cdot}85 \times 10^{-4}$ M) each and to a similar extent retard the rate of glucose-uptake from oxygenated, phosphate-buffered medium by normal rat diaphragm. With diaphragms from chronically morphinised rats, the separate effects of both hormones are quite different, adrenaline being without effect while hydrocortisone has the opposite effect and accelerates the rate of glucose-uptake.

These differences are masked when the two hormones are added together, since adrenaline depresses glucose-uptake in the hydrocortisone-stimulated, chronically morphinised diaphragm and hydrocortisone stimulates glucose-uptake in the adrenaline-depressed, normal diaphragm.

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

State of rat	Number of rats	Control	Experiment	Difference
			+ Adrenaline	
Normal	(6)	154 ± 14	34 ± 12	−120 ± 13 ($P \ll 0{\cdot}001$)
Chronically morphinised	(8)	189 ± 56	188 ± 52	−1 ± 39
			+ Hydrocortisone	
Normal	(6)	193 ± 41	52 ± 27	−141 ± 27 ($P < 0{\cdot}01$)
Chronically morphinised	(7)	228 ± 29	325 ± 30	+97 ± 35 ($P < 0{\cdot}05$)
			+ Adrenaline + Hydrocortisone	
Normal	(8)	252 ± 38	132 ± 44	−120 ± 28 ($P < 0{\cdot}01$)
Chronically morphinised	(8)	196 ± 52	103 ± 44	−93 ± 12 ($P < 0{\cdot}001$)
		+ Hydrocortisone	+ Adrenaline + Hydrocortisone	
Normal	(6)	223 ± 36	140 ± 25	−85 ± 25 ($P = 0{\cdot}02$)
Chronically morphinised	(8)	285 ± 38	150 ± 24	−135 ± 42 ($P < 0{\cdot}02$)
		+ Adrenaline	+ Adrenaline + Hydrocortisone	
Normal	(6)	134 ± 29	207 ± 40	+73 ± 23 ($P < 0{\cdot}05$)
Chronically morphinised	(8)	203 ± 21	187 ± 28	−16 ± 28

Hemi-diaphragms were incubated at pH 7·4 and 37° for 2 hr in oxygenated Krebs-Ringer-phosphate (2·0 ml) containing glucose (0·15%) ± added hormone (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.

Effects of adrenaline and hydrocortisone on glycogen metabolism in isolated diaphragm of normal and of chronically morphinised rats

In the experiments here reported (Table 2) the balance between glycogenesis and glycogenolysis in isolated diaphragms of both normal and chronically morphinised rats when incubated in oxygenated, phosphate-buffered medium containing glucose is in favour of glycogenesis. Addition of either adrenaline or hydrocortisone, or both, each in $3{\cdot}85 \times 10^{-4}$ M concentration, changes the balance so that glycogenolysis predominates. Greater effects are obtained with diaphragm from chronically morphinised animals, especially with adrenaline, but there is no marked difference between normal and chronically morphinised tissue in the nature of the effects of the hormones on glycogen metabolism.

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

State of rat	Number of rats	Control	Experiment	Difference
			+ Adrenaline	
Normal	(6)	+33 ± 6	−8 ± 2	−41 ± 6·5 ($P < 0{\cdot}002$)
Chronically morphinised	(8)	+28 ± 10	−55 ± 10	−83 ± 16 ($P < 0{\cdot}001$)
			+ Hydrocortisone	
Normal	(6)	+17 ± 3	−13 ± 3	−30 ± 4 ($P < 0{\cdot}001$)
Chronically morphinised	(8)	+17 ± 10	−30 ± 10	−47 ± 9 ($P < 0{\cdot}002$)
			+ Adrenaline + Hydrocortisone	
Normal	(8)	+28 ± 3	−37 ± 4	−65 ± 5 ($P < 0{\cdot}001$)
Chronically morphinised	(6)	+69 ± 12	−29 ± 4	−98 ± 9 ($P < 0{\cdot}001$)
		+ Hydrocortisone	+ Adrenaline + Hydrocortisone	
Normal	(4)	−26 + 7	−39 ± 8	−13 ± 2 ($P < 0{\cdot}01$)
Chronically morphinised	(8)	+5 ± 4	−22 + 5	−27 ± 4 ($P < 0{\cdot}001$)
		+ Adrenaline	+ Adrenaline + Hydrocortisone	
Normal	(6)	−16 ± 5	−21 ± 5	−5 ± 1 ($P < 0{\cdot}02$)
Chronically morphinised	(8)	−32 ± 4	−35 ± 5	−3 ± 3

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 (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 g wet tissue per hr.

Effects of adrenaline and hydrocortisone on lactate accumulation in isolated diaphragm of normal and of chronically morphinised rats

Both adrenaline and hydrocortisone tend to increase the rate of lactate accumulation by isolated diaphragm in oxygenated, phosphate-buffered medium containing

glucose and a significant increase occurs when both hormones are added together (Table 3) and increased glycogenolysis (Table 2) is not accompanied by an increased uptake of oxygen (Table 4).

No appreciable difference between normal and chronically morphinised tissue is revealed by the effects of either or both hormones on the rates of lactate production.

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

State of rat	Number of rats	Control	Experiment	Difference
			+ Adrenaline	
Normal	(6)	77 ± 5	80 ± 4	+3 ± 5
Chronically morphinised	(8)	79 ± 4	86 ± 4	+7 ± 4
			+ Hydrocortisone	
Normal	(6)	93 ± 10	109 ± 9	+16 ± 10
Chronically morphinised	(8)	85 ± 4	98 ± 3	+13 ± 3 ($P < 0.01$)
			+ Adrenaline + Hydrocortisone	
Normal	(8)	107 ± 3	138 ± 5	+31 ± 4 ($P < 0.001$)
Chronically morphinised	(6)	82 ± 2	108 ± 4	+26 ± 4 ($P < 0.002$)
		+ Hydrocortisone	+ Adrenaline + Hydrocortisone	
Normal	(10)	132 ± 7	152 ± 11	+20 ± 5 ($P < 0.01$)
Chronically morphinised	(8)	87 ± 3	105 ± 6	+18 ± 6 ($P < 0.02$)
		+ Adrenaline	+ Adrenaline + Hydrocortisone	
Normal	(6)	89 ± 5	107 ± 5	+18 ± 4 ($P < 0.01$)
Chronically morphinised	(8)	78 ± 2	107 ± 2	+29 ± 5 ($P < 0.001$)

Hemi-diaphragms were incubated at pH 7·4 and 37° for 2 hr in oxygenated Krebs-Ringer-phosphate (2·0 ml) containing glucose (0·15%) ± added hormone (each in final concentration, $3{\cdot}85 \times 10^{-4}$ M). In each experiment, half the excised diaphragm served as the 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.

Effects of adrenaline and hydrocortisone on uptake of oxygen by isolated diaphragm of normal and of chronically morphinised rats

Adrenaline stimulates and hydrocortisone depresses respiration by isolated diaphragm in oxygenated phosphate-buffered medium containing glucose and no appreciable change in the rate of respiration results when both hormones are added together in equimolecular concentration (Table 4).

Apart from a suggestion that the chronically morphinised tissue may be slightly more sensitive to adrenaline, no appreciable difference between normal and chronically morphinised tissue is revealed by the effects of either or both hormones on the rates of oxygen consumption.

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

State of rat	Number of rats	Control	Experiment	Difference
			+ Adrenaline	
Normal	(10)	919 ± 30	1,015 ± 26	+96 ± 22 ($P < 0.002$)
Chronically morphinised	(10)	1,089 ± 35	1,205 ± 50	+113 ± 41 ($P < 0.05$)
			+ Hydrocortisone	
Normal	(6)	993 ± 78	707 ± 50	−286 ± 85 ($P < 0.02$)
Chronically morphinised	(8)	1,138 ± 65	863 ± 62	−275 ± 38 ($P < 0.001$)
			+ Adrenaline + Hydrocortisone	
Normal	(8)	1·035 ± 60	979 ± 76	−56 ± 37
Chronically morphinised	(8)	1,076 ± 31	1,053 ± 64	−23 ± 60
		+ Hydrocortisone	+ Adrenaline + Hydrocortisone	
Normal	(10)	819 ± 66	959 ± 91	+140 ± 36 ($P < 0.01$)
Chronically morphinised	(7)	1,087 + 57	1,270 ± 95	+183 ± 63 ($P < 0.05$)
		+ Adrenaline	+ Adrenaline + Hydrocortisone	
Normal	(13)	1,132 ± 68	964 ± 84	−168 ± 33 ($P < 0.001$)
Chronically morphinised	(10)	1,385 ± 56	1,257 ± 63	−128 ± 56 ($P < 0.05$)

Hemi-diaphragms were incubated with shaking in oxygenated Krebs-Ringer-phosphate (2·0 ml) containing glucose (0·15%) ± added hormone (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.

DISCUSSION

Whereas both adrenaline and hydrocortisone influence various stages of intracellular carbohydrate metabolism in muscle, as compared in the present work by measurement of their separate and combined effects on glycogen metabolism, lactate accumulation, and respiration, it is only in their effects on the glucose uptake mechanism that marked differences between normal and chronically morphinised tissue are observed. This gives added support to the view, expressed in a previous publication,[2] that morphine exerts its major influence at the cell membrane and that this is the site where changes induced by chronic morphinisation specifically involve mechanisms that are subject to hormonal influence.

With regard to intracellular metabolism, the results of our experiments are in general agreement with the commonly accepted views on the separate effects of the two hormones. These have been extensively reviewed.[3–7] Apart from the opposing effects on respiration, there is no indication from the experiments in which both hormones were used that one hormone either potentiates or antagonises the action of the other. The apparently synergistic effect on lactate production can be explained

in terms of their separate effects, adrenaline stimulating glycogenolysis and glycolysis, hydrocortisone preventing a compensatory rise in respiration. These observations favour the view that the steroid causes a diminution in glycogen content by inhibiting synthesis rather than by accelerating glycogenolysis. In general, the separate and combined effects of the hormones on chronically morphinised diaphragm are similar to those on normal diaphragm, though there is a suggestion that the chronically morphinised tissue is somewhat more sensitive, especially to adrenaline. The effect of adrenaline on glycogenolysis is significantly greater ($P < 0{\cdot}05$) in the diaphragm from chronically morphinised rats, but such differences are slight when compared with the effects on glucose-uptake.

With regard to the effects of hydrocortisone and adrenaline on the glucose-transport system in muscle there is no general agreement and many conflicting reports are discussed in the reviews.[3-7] Ramey and Goldstein[4] have pointed out that, whereas most investigators have studied the effects of the steroids and adrenalines on carbohydrate metabolism separately, they appear to operate largely as a functional unit physiologically and that many actions attributed to the steroids may be ascribed, in effect, to adrenaline. Also it is apparent that the effect of a hormone may vary according to its concentration, both absolute and in relation to a variety of other substances and, as our work with chronically morphinised animals demonstrates, it may vary according to the state of the tissue upon which it acts.

We find, for example, that whereas hydrocortisone in $7{\cdot}7 \times 10^{-4}$ M concentration, as used in our previous experiments with morphine,[2] has no effect on glucose-uptake by isolated diaphragm of either normal or chronically morphinised rats, it has very marked effects in the lower concentration used in the present work, and, as reported in a preliminary communication,[8] the effect on the tissue from a chronically morphinised animal is quite different from that on the tissue from a normal animal. These effects are changed by adrenaline. Thus, hydrocortisone depresses the uptake of glucose by normal diaphragm, but accelerates uptake by chronically morphinised diaphragm. In the presence of adrenaline, hydrocortisone accelerates glucose-uptake in normal diaphragm but not in chronically morphinised diaphragm. The response to addition of hydrocortisone by normal tissue in the presence of added adrenaline is thus similar to that of chronically morphinised tissue in the absence of added adrenaline.

Differences between normal and chronically morphinised tissue in response to adrenaline are observed also, and hydrocortisone modifies the effects of adrenaline. Adrenaline depresses glucose-uptake by the normal diaphragm but is without effect on glucose-uptake by the chronically morphinised diaphragm. Addition of hydrocortisone apparently restores adrenaline-sensitivity to the chronically morphinised diaphragm and the response to addition of adrenaline by chronically morphinised tissue in the presence of hydrocortisone is thus similar to that of normal tissue in the absence of added hydrocortisone.

It is of interest to compare this morphine-induced decrease in adrenaline-sensitivity of the glucose-transport system with the morphine-induced increase in adrenaline-sensitivity of the intracellular systems. An increased permeability of the membrane to adrenaline might explain this. A variety of factors are known to influence sensitivity to adrenaline. Herman and Ramey[9] have found that glucose-uptake by rat diaphragm in a bicarbonate-buffered medium is not affected by adrenaline and that the depressant

effect of adrenaline in a phosphate-buffered medium is abolished by raising the magnesium content of the medium. This last observation is of interest in view of the possibility, the evidence for which has been reviewed by Tepperman and Tepperman,[6] that hormones may act as sequestering agents for ions such as Mg^{++} and thus variably influence the distribution of ions involved in the activity of certain enzymes and in the controlling mechanisms of membrane-transport. Possibly, morphine acts in this way and it may be significant, not merely circumstantial, that raising the magnesium content of the incubation medium has the same effect on adrenaline-sensitivity of the diaphragm as prior treatment of the animal with morphine. We are proceding to investigate this possibility and to study the combined effects of adrenaline and morphine in experiments analogous to those with hydrocortisone and morphine, reported in a previous paper,[2] and with adrenaline and hydrocortisone as reported in the present work.

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