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# GLYCOGEN CONTENT AND PHOSPHORYLASE ACTIVITY IN LIVER AND SKELETAL MUSCLE OF NORMAL AND CHRONICALLY MORPHINIZED RATS

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**Abstract**—The glycogen content and phosphorylase activity of liver, hindlimb muscle and diaphragm from normal and chronically morphinized rats have been determined. There is a sharp decline of glycogen in liver, but not in skeletal muscle, from chronically morphinized rats. Glycogen phosphorylases *a* and *b* are reduced in both liver and skeletal muscle. There is a relative increase of the proportion of phosphorylase-*b* in the tissues of morphinized animals. The increase of the ratio of phosphorylase-*b*–phosphorylase-*a* may be related to the inhibition of hormonal action and of activation of adenyl cyclase caused by the drug.

IT IS well documented that phosphorylase has an important role to play in the regulation of carbohydrate metabolism through its control of glycogenolysis[1] and that this activity is affected by hormones such as adrenaline and glucagon and by $Ca^{2+}$ and $Mg^{2+}$.[2–5] It has also been established that repeated administration of morphine can bring about disturbances in endocrine secretions[6,7] and can induce changes in cell membranes[7–10] and in the intracellular distribution of $Na^+$, $K^+$, $Ca^{2+}$ and $Mg^{2+}$.[8]

The question therefore arises as to what effect chronic morphinization might have on the glycogenolytic system, through its effect on phosphorylase activity. In an attempt to answer this question the glycogen content and the phosphorylase activity of liver and skeletal muscle of normal and chronically morphinized rats have been determined.

## MATERIALS AND METHODS

*Animals.* Female rats weighing about 160 g received daily injections of morphine sulphate (0·3 mg/100 g body wt) for 1 and 5 weeks, to induce morphinization. Control animals received daily injections of normal saline for the same periods of time. The animals were killed by decapitation 24 hr after the final injection. Food was withheld for the final 15 hr.

*Chemicals.* Glucose-1-phosphate and Adenosinemonophosphate (AMP) were obtained from Sigma Company and glycogen from British Drug Houses. Glycogen was purified by stirring two-thirds of its weight of activated charcoal together with the glycogen in distilled water for 1 hr, filtering and precipitating with 1 vol. of 95 per cent ethanol and washing with ethanol and ether.[9]

*Assay of phosphorylase.* Immediately after the animal was killed, 0·5 g of liver 0·5 g of hindlimb muscle and 0·2 g of diaphragm were taken and homogenized with 10 ml of cold extracting reagent of the following composition: NaF 0·02 M, EDTA 0·005 M,

TABLE 1. GLYCOGEN CONTENTS OF LIVER AND SKELETAL MUSCLES FROM NORMAL AND CHRONICALLY MORPHINIZED RATS

| State of rats | Average body wt (g) | Average liver wt (g) | Glycogen content of tissue mg/g of tissue | | | |
|---|---|---|---|---|---|---|
| | | | Liver | P | Hindlimb muscle | Diaphragm |
| | | | ± S. E. M. | | ± S. E. M. | ± S. E. M. |
| Normal | 181 (5) | 5·57 (5) | 70·4 ± 3·18 (4) | < 0·005 | 5·14 ± 0·34 (4) | 2·59 ± 0·83 |
| Morphinized 1 week | 178 (4) | 5·15 (4) | 36·47 ± 11·93 (4) | < 0·005 | 5·40 ± 0·39 (4) | 2·07 ± 0·31 (4) |
| Morphinized 5 weeks | 176 (6) | 5·50 (6) | 22·28 ± 3·76 (5) | | 4·96 ± 0·44 (7) | 1·99 ± 0·48 (7) |

Values in parentheses indicate the number of rats in each group.

cysteine–HCl 0·03 M, Na-glycerol-phosphate 0·04 M, pH adjusted to 8·6. For diaphragm homogenate, the tissue was ground with chemically pure sand and the extracting solution. The homogenates were centrifuged for 40 min at 4000 *g*. The supernatants were used for the enzyme assay.

Phosphorylase activity was determined by measuring the release of inorganic phosphate from glucose-1-phosphate in the presence of glycogen.[17,25] Tubes containing NaF 80 $\mu$M, glycogen 0·1 ml (20 mg/ml), buffer pH 7·0 (with the same composition as the extracting solution), K-glucose-1-phosphate 60 $\mu$M, 5-AMP 10 $\mu$M in a total volume of 1·0 ml, and 0·1 ml of the homogenate were incubated at 37° for 15 min, with 1·0 ml of 10% TCA added to stop the reaction. Generally, two series of tubes were incubated, one for phosphorylase (*a*) with no addition of AMP and the other for total phosphorylase (*a* + *b*) with the addition of 5-AMP. After centrifugation of the tubes, 0·2 ml of the supernatant was taken for phosphate determination.[10] To another portion of the homogenate (0·2 ml), 1·0 ml of 10% TCA and 0·8 ml of water were added, and the precipitate was washed with 5% TCA. The protein precipitate was resuspended in water and determined by biuret reaction.[11]

*Determination of glycogen.* Glycogen was isolated by the method of Good, Kramer and Somogyi[12] and determined by the Anthrone method.[13]

## RESULTS AND DISCUSSION

*Glycogen content of liver, hindlimb muscle and diaphragm of normal and chronically morphinized rats.* The results given in Table 1 show that normal and chronically morphinized rats differ markedly in glycogen content of liver, but are not significantly different in body weight. Evidently, the lower glycogen of the morphinized animals cannot be attributed to nutritional influences. It may be explained as a result of either an increase in the rate of glycogenolysis, a decrease in the rate of glycogenesis, or both. Since the phosphorylase activity of the morphinized liver is reduced (Table 2), it is considered more likely that the change in glycogen may be due mainly to its decreasing rate of synthesis. It has been previously observed that morphine, *in vitro*, inhibits the uptake of glucose by the diaphragm of chronically morphinized rats and, consequently, the rate of glycogen synthesis.[14] The same action may affect morphinized liver, resulting in the inhibition of the synthesis of glycogen.

In addition to the direct action of morphine, hormonal effects may be contributory to a decrease in the rate of glycogenesis. As the liver glycogen is reduced whereas the muscle glycogen is not (Table 1), this suggests that glucagon secretion may have increased. Glucagon causes depletion of liver glycogen but has no effect on muscle.[15] This is because it stimulates adenyl cyclase of the liver, thus promoting the breakdown of glycogen and inhibiting the synthesis.

*Phosphorylase activities of liver, hindlimb muscle and diaphragm of normal and chronically morphinized rats.* It can be seen from Table 2 that after chronic morphinization all three tissues studied show a decrease in phosphorylase activity. The enzyme activity measured with the addition of AMP represents the total phosphorylase activity of both *a*- and *b*-forms whereas when AMP is absent from the medium only the *a*-form is active. After the injection of morphine for 1 week, there was a significant decrease in phosphorylase-*a* in the liver whereas its total phosphorylase remained almost the same as before. This suggests that there was no net change in synthesis of the enzyme, but a conversion of phosphorylase-*a* to phosphorylase-*b* may have taken

TABLE 2. PHOSPHORYLASE ACTIVITIES OF LIVER AND SKELETAL MUSCLE FROM NORMAL AND CHRONICALLY MORPHINIZED RATS

| State and No. of rats | Phosphorylase activities of liver γP/mg protein/10 min | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | −AMP (*a*)* | P | | AMP (*a* + *b*)* | P | *b* | *b*/*a* | P |
| Normal (5) | 5·05 ± 0·98 | <0·01 | <0·025 | 7·46 ± 0·6 | 0·001 | 2·41 | 0·41 ± 0·25 | 0·05 |
| Morphinized 1 week (4) | 3·32 ± 0·47 | | | 6·07 ± 0·19 | | 1·65 | 0·80 ± 0·11 | |
| Morphinized 5 weeks (5) | 2·47 ± 0·19 | | | 4·07 ± 0·39 | | 1·60 | 0·65 ± 0·05 | |

Phosphorylase activities of skeletal muscle γP/mg protein/10 min

| State and No. of rats | Hindlimb muscle | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | −AMP (*a*)* | P | | +AMP (*a* + *b*)* | P | *b* | *b*/*a* | P |
| Normal (5) | 17·63 ± 3·67 | <0·05 | <0·001 | 109·10 ± 4·39 | <0·005 | 81·5 | 4·6 ± 0·70 | <0·05 |
| Morphinized 1 week (4) | 20·0 ± 1·44 | | | 90·98 ± 1·78 | | 70·98 | 3·60 ± 0·45 | |
| Morphinized 5 weeks (5) | 9·57 ± 1·45 | | | 62·24 ± 2·71 | | 52·43 | 5·80 ± 1·0 | |

Phosphorylase activities of skeletal muscle γP/mg protein/10 min

| State and No. of rats | Diaphragm | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | —AMP (*a*)* | P | | +AMP (*a* + *b*)* | P | *b* | *b*/*a* | P |
| Normal (5) | 3·78 ± 1·22 | 0·005 | | 61·68 ± 9·74 | 0·0005 | 57·90 | 15·4 ± 9·5 | <0·01 |
| Morphinized 1 week (4) | 5·23 ± 0·23 | | | 61·58 ± 2·42 | | 56·53 | 10·9 ± 2·2 | |
| Morphinized 5 weeks(5) | 1·38 ± 0·41 | | | 37·54 ± 2·84 | | 36·16 | 26·4 ± 3·2 | |

(*a*)* Active phosphorylase. (*a* + *b*)* Total phosphorylase.

place. After a longer period of morphine administration, both active and total phosphorylases of skeletal muscle and liver were lowered significantly. However, there is a general increase in the ratio of phosphorylase-*b* to phosphorylase-*a* in these tissues. It is clear that the physiological conditions created by morphinization are more favourable to the form-*b* than the form-*a* of phosphorylase.

The relative increase of the proportion of phosphorylase-*b* in the cells of morphinized animal may be related to the inhibition of hormonal action and enzyme activation caused by the drug. It is known that activating of phosphorylase-*b* to phosphorylase-*a* is catalyzed by phosphorylase kinase. This process is facilitated by cyclic 3′,5′-AMP, which is derived from Adenosinetriphosphate (ATP) by adenyl cyclase.[15] Moreover, adenyl cyclase is located in cell membrane[16] and is stimulated by adrenaline.[17] It has been noted that when a hormone produces a physiological reaction, a hormone

receptor bond is formed,[18] and that for the formation of an adrenaline-receptor binding of liver plasma membrane, the benzene ring and two OH groups of the ring are essential.[18] It is the binding of adrenaline to the membrane that causes an increase in the activity of the membrane bound enzyme, adenyl cyclase. However, as the morphine molecule also possesses a benzene ring and two OH groups and its site of action is similarly in the cell membrane,[14,19,20] it may probably compete with adrenaline for the binding site in membranes so as to eliminate the hormone action which stimulates adenyl cyclase. Thus, the process for activating phosphorylase-*b* may become inhibited, resulting in an increase in this form of enzyme.

Further, as suggested by Rasmussen,[21] certain hormones may interact with the receptor in the membrane to simultaneously increase $Ca^{2+}$ permeability and adenyl cyclase activation. Since the concentration of $Ca^{2+}$ is critical for the interplay of hormone binding and enzyme activation,[22] it appears likely that any disturbance causing a change in $Ca^{2+}$ concentration would affect the activation of adenyl cyclase. Chronic morphinization may well provide such a disturbance and bring about a change of $Ca^{2+}$ concentration, for morphine has been previously found to be capable of causing redistribution of Ca and other inorganic ions in intracellular particles.[8] Then, with adenyl cyclase inactivated, activation of phosphorylase-*b* may be inhibited.

To conclude, the present study lends further support to the view of previous workers that morphine induces changes in the membrane, disturbs hormone secretion and function, changes the concentration and distribution of some inorganic ions, and affects enzyme activities and then metabolic processes. The drug simulates a hormone and is indispensable in a newly established hormone balance in the control of metabolism. This may be the basis of addiction.

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