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Factors Influencing Nonspecific Binding of Glucocorticoids in Myocardial Tissue[1] (29234)

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The use of the synthetic glucocorticoids, methylprednisolone, and dexamethasone in the treatment of acute myocardial infarction has recently received renewed attention (1–5). The binding and transport characteristics of glucocorticoids at physiological levels have been studied in the liver (6, 7), kidneys (8–10), and lung (11, 12). However, very few studies have been done on the heart (13, 14), and no studies have been conducted at the pharmacological doses reported to be necessary for the stabilization of myocardial cellular membranes (4, 15, 16) during ischemic states such as acute myocardial infarction. The purpose of this study is to characterize the uptake of the two most widely used glucocorticoids, (i.e., dexamethasone and methylprednisolone) by the myocardium at the pharmacological levels used in the treatment of acute myocardial infarction and cardiogenic shock.

Methods. [1,2-^{3}H]Dexamethasone (^{3}H-DEXA, 25 Ci/mmole) and [^{3}H]sorbitol (1.7 Ci/mmole) were obtained from Amersham Searle Corporation, and methylprednisolone-^{3}H-21-sodium succinate (^{3}H-MP, 0.0497 Ci/mmole) from New England Nuclear Corporation. Unlabeled dexamethasone sodium phosphate (Decadron) was supplied by Merck Sharp and Dohme, and methylprednisolone sodium succinate (Solu-Medrol) by the Upjohn Company. Potassium thiocyanate, *p*-chloromercuribenzoate, and 2,4-dinitrophenol were purchased from the Sigma Chemical Company. All other chemicals were of reagent grade.

Mongrel cats of either sex weighing 2.5 to 3.5 kg were anesthetized with sodium pentobarbital (30 mg/kg), and the hearts were removed via a midventral thoracotomy and placed in ice-cold Krebs-Henseleit bicarbonate buffer, pH 7.4, containing 10 m*M* glucose (K–H buffer). Subsequent preparations of the myocardium were performed at 4° in a cold room. The hearts were flushed with 100 ml of cold K–H buffer by canulating the aorta and removing most of the blood from the coronary bed. The left ventricle was dissected and sliced in a Stadie-Riggs tissue slicer at a thickness of 0.5 mm. The myocardial slices, each of which weighed 100–200 mg, were placed in incubation flasks containing fresh K–H buffer. The tissue to medium ratio did not exceed 1/50 (w/v).

Measurement of glucocorticoid uptake. The slices were incubated in a Dubnoff metabolic shaker at 37 or 0° under atmosphere of 95% O_2 plus 5% CO_2. The concentrations of DEXA and MP were 0.171 m*M* (sp act 585 μCi/mmole) and 0.805 m*M* (sp act 124 μCi/mmole), respectively. These concentrations used were equivalent to dose levels of 6 mg DEXA/kg of body weight and 30 mg MP/kg of body weight, which are doses usually employed in the treatment of acute myocardial infarction and shock.

Samples were removed from the K–H medium for measurement of radioactivity at times ranging from 0 (15 sec) to 180 min. In the series of experiments in which the effect of various inhibitors was studied, slices were preincubated with the inhibitors for 30 min. Following preincubation, glucocorticoid was added to the medium and the incubation was continued for an additional 60 min. A control flask receiving no inhibitors was included in each experiment. The concentrations of inhibitors used were (a) 1.2×10^{-2}

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M *p*-chloromercuribenzoate (PCMB), (b) 0.1 *M* potassium thiocyanate (KSCN), (c) 1 $\times 10^{-2}$ *M*, 2,4 dinitrophenol (DNP), and (d) 95% N_2 plus 5% CO_2.

Tissue preparation for liquid scintillation counting. After incubation, the myocardial slices were carefully blotted on Whatman #4 filter paper to remove excessive moisture, weighed, and placed in scintillation counting vials containing 10 ml of Multisol counting solution (Isolab, Inc.). The slices were them homogenized with a Polytron PT-10 tissue homogenizer for 60 sec. This procedure homogenized the tissue adequately and yielded a counting efficiency of approximately 30%. Counting of radioactivity was done on a Tri-Carb Packard Liquid-Scintillation Spectrometer, disintegrations per minute being determined by an external standard method.

Determination of extracellular space. Extracellular space of the myocardial slices was determined by labeling the tissue with 10 m*M* sorbitol (sp act 50 μCi/mmole) in K-H buffer for various times up to 180 min. Radioactivity of the labeled slices was determined as above. The glucocorticoid uptake rates were then corrected for the extracellular fluid space.

Statistics. All data were compared using the Student's *t* test for significance.

Results. Extracellular space of cardiac slices. Cat left-ventricular myocardial slices, incubated in K-H with 10 m*M* [^{3}H]sorbitol and using the identical protocol as that for steroid uptake at 37 and 0°, showed that extracellular space accounted for 45–53% of the calculated steroid uptake. All subsequent steroid uptake data have been corrected for extracellular space.

Glucocorticoid uptake at 0 and 37°. Figure 1 shows the uptake of DEXA (upper panel) and MP (lower panel) by myocardial slices at temperatures of 0 and 37° over a period of 180 min. Both glucocorticoids showed a significant increase in uptake over this time. After 60 min of incubation, steroid uptake plateaued at 0.21 μmole of DEXA/g of tissue and 0.95 μmole of MP/g of tissue.

With either steroid, the uptake rates and total amounts of glucocorticoid in the myocardial cell were significantly ($P < 0.001$) reduced at 0° compared to 37°. Only about

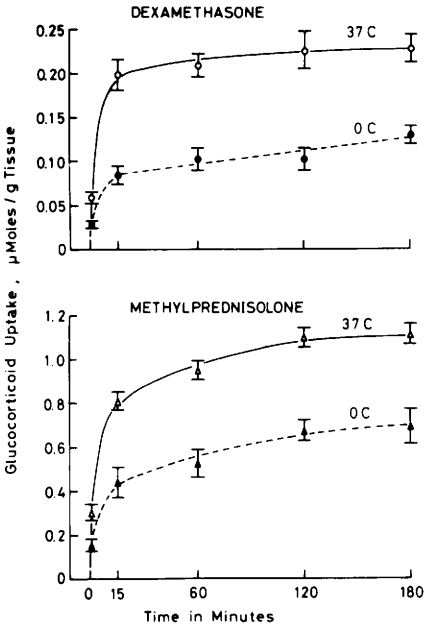

FIG. 1. Time-course of uptake of dexamethasone and methylprednisolone by myocardial slices. The myocardial uptake of radioactivity was measured from 0 to 180 min, and corrected for extracellular space of the myocardial slices. Experimental points represent mean of six experiments, and bars ± SEM.

50% of the 37° values were taken up at 0°. This occurred at every time point measured.

Steroid uptake in response to varying extracellular concentrations. To determine if the steroid uptake in myocardial slices was approaching saturation, heart slices were incubated for 60 min in the presence of varying concentrations of DEXA or MP. The results of these experiments are shown in Fig. 2. Neither steroid appears to saturate cardiac tissue at the concentrations used, and appears to have a linear relationship between uptake and extracellular concentration. Thus, the myocardial cell is apparently capable of taking up large quantities of DEXA and MP, and the intracellular concentration is dependent upon the extracellular concentration of the glucocorticoid.

Response of myocardial cells to glucocorticoid uptake in the presence of inhibitors. To determine whether the uptake of DEXA and MP in heart slices is an energy-dependent phenomenon, slices were incubated in the presence of various metabolic inhibitors. The results of these experiments are sum-

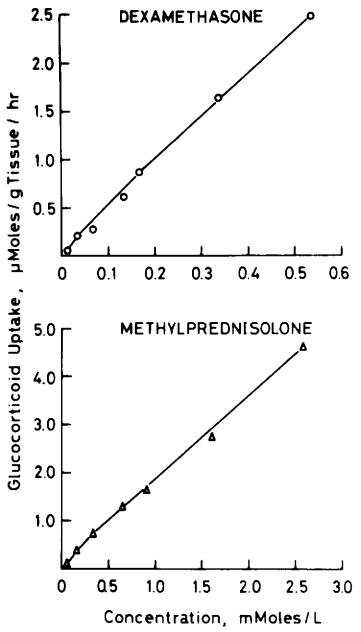

FIG. 2. Uptake of dexamethasone and methylprednisolone by myocardial slices as a function of external glucocorticoid concentration. Glucocorticoid concentrations in the incubation medium were varied over ranges 0.01 to 0.54 m*M* (DEXA) and 0.06 to 2.58 m*M* (MP) at the same specific activity. Incubation was carried out for 60 min at 37° under 95% O_2 + 5% CO_2. Results of one representative experiment are shown.

marized in Fig. 3. Incubation of myocardial slices in the presence of PCMB ($1.2\ M \times 10^{-2}\ M$), Nitrogen (95% N_2 and 5% CO_2), and DNP ($1 \times 10^{-2}\ M$) showed no significant differences in the glucocorticoid uptake compared to control values. However, KSCN at 0.1 *M* showed a significant decrease in glucocorticoid uptake compared to controls.

Steroid uptake at various pH values. Figure 4 illustrates the response of glucocorticoid uptake in heart slices at various pH values. Both DEXA and MP appear to have an optimal cardiac uptake between pH 7.3–7.4. Uptake of both steroids exhibit an abrupt decline of 20% or more at values above pH 7.4.

Conclusions. The sorbitol space calculated by our experiments were slightly higher than the 42 to 46% reported by Neely *et al.* (17) in rat hearts. However, this difference may be due to a larger surface to volume ratio encountered in myocardial slices compared to the Langendorff or working heart preparations used by these investigators.

The slightly reduced uptake of steroid in the presence of KSCN, may not be of primary significance since the high concentrations of the inhibitor used in this study may compromise the integrity of the cell membrane. At KSCN concentrations at 0.05 *M*, we found no significant changes in the steroid uptake.

Concentrations of steroids used in this study are comparable to the concentrations used by various investigators in studying the protective effect of glucocorticoids in various shock states (2, 16). This high dose precluded any observations on the kinetics of specific binding proteins in the heart as reported by Funder *et al.* (9, 13) and Ballard *et al.* (11). These data represent characteristics of the nonspecific binding sites in the

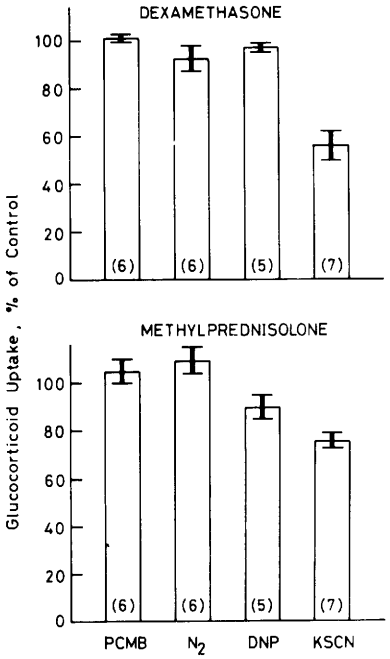

FIG. 3. Effect of *p*-chloromercuribenzoate, nitrogen, 2,4-dinitrophenol, and potassium thiocyanate on myocardial uptake of glucocorticoids. Myocardial slices were incubated for 60 min at 37° in presence of various inhibitors: *p*-chloromercuribenzoate (PCMB), $1.2 \times 10^{-2}\ M$; 95% N_2–5% CO_2; 2,4-dinitrophenol (DNP), $1 \times 10^{-2}\ M$; and potassium thiocyanate (KSCN), 0.1 *M*. Each bar represents mean ± SEM. Numbers in parentheses indicate the number of experiments.

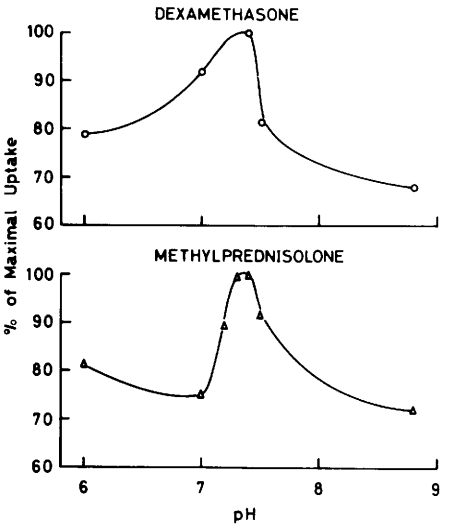

FIG. 4. Influence of pH on myocardial uptake of glucocorticoids. The final pH and uptake of either DEXA or MP were measured at 60 min incubation at 37°. The values are plotted as percentage of the maximal uptake observed against the final pH in the incubation media. Results of one typical experiment are shown for each glucocorticoid.

myocardial cell. The lack of saturation kinetics coupled with the evidence that no apparent energy is required in effecting transport of the steroid across cell membranes is consistent with the findings of Munck *et al.* (18), who found that the nonspecific binding fraction of glucocorticoids in thymus cells could be characterized by a lack of polarity, saturation, or energy requirement. Additionally, they found that at 37°, thymus cells were freely permeable to glucocorticoids.

No studies have been reported on the temperature effect of the nonspecific binding kinetics of glucocorticoids in the cell, but Baxter and Tomkins (19) have reported that at 0°, DEXA binding and dissociation are very slow and that at 37°, binding increases appreciably.

To our knowledge, this is the first study to characterize physical factors that modify the binding of pharmacologic doses of glucocorticoids in myocardial cells. Myocardial tissue has the capacity to accumulate large quantities of glucocorticoids with a significant fraction bound to cell membranes. This uptake does not require energy but is temperature- and pH-dependent. All of the isotope label taken up by heart slices may not be native glucocorticoid. However, since only 10% of labeled glucocorticoid is metabolized by 90 min in the intact heart (20), one would expect at least 90% of the label to be intact glucocorticoid after incubation for 60 min with heart slices.

Summary. Myocardial slices from the left ventricle of cat hearts were incubated in Krebs–Henseleit buffer containing 10 m*M* glucose which were gassed with 95% O_2 and 5% CO_2. Tritiated dexamethasone (DEXA 0.171 m*M*) or methylprednisolone (MP 0.805 m*M*) was added under varying conditions of temperature, pH, and in the presence of various metabolic inhibitors. Glucocorticoid uptake by myocardial tissue was found to be temperature-dependent, plateauing after 60 min of incubation at 0.21 μmole of DEXA/g of tissue and 0.95 μmole of MP/g of tissue at 37°. Steroid uptake was retarded about 50% at 0°. Increasing the concentration of either steroid resulted in a linear increase in the uptake of that steroid. Incubation in the presence of metabolic or SH-inhibitors produced no significant changes in glucocorticoid uptake. Optimal glucocorticoid uptake occurred between pH 7.3–7.4. Thus, myocardial cells appear to be able to take up large quantities of glucocorticoids, but the uptake process does not appear to be energy-dependent.

1. Libby, P., Maroko, P. R., Bloor, C. M., Sobel, B. E., and Braunwald, E., J. Clin. Invest. **52,** 599 (1973).
2. Vyden, J. D., Nagasawa, K., Rabinowitz, B., Parmley, W. W., Tomoda, H., Corday, E., and Swan, H. J. C., Amer. J. Cardiol. **34,** 677 (1974).
3. Morrison, J., Reduto, L., Maley, T., and Gulotta, S., Amer. J. Cardiol. **35,** 158 (1975).
4. Spath, J. A., Jr., Lane, D. L., and Lefer, A. M., Circ. Res. **35,** 44 (1974).
5. Spath, J. A., Jr., and Lefer, A. M., Amer. Heart J. **90,** 50 (1975).
6. Litwack, G., Morey, K. S., and Kitterer, B., *in* "Effects of Drugs on Cellular Control Mechanisms" (B. R. Rabin, ed.), p. 105. MacMillan, New York (1972).
7. Beato, M., Schmid, W., Braendel, W. B., Biesewig, D., and Sekeris, C. E., *in* "Advances in the Biosciences 7" (G. Raspe, ed.), p. 349. Pergamon Press, Gieweg, Oxford (1970).

8. Rousseau, G., Baxter, J. D., and Tomkins, G. M., J. Molec. Biol. **67** (1972).
9. Funder, J. W., Feldman, D., and Edelman, I. S., Endocrinology **92,** 1005 (1973).
10. Feldman, D., Funder, J. W., and Edelman, I. S., Endocrinology **92,** 1429 (1973).
11. Ballard, P. L., and Ballard, R. A., Proc. Nat. Acad. Sci., USA **69,** 2668 (1972).
12. Giannopoulos, G., J. Biol. Chem. **248,** 3876 (1973).
13. Funder, J. W., Duval, D., and Meyer, P., Endocrinology **93,** 1300 (1973).
14. Ballard, P. L., Baxter, J. D., Higgins, S. J., Rousseau, G. G., and Tomkins, G. M., Endocrinology **94,** 998 (1974).
15. Weissmann, G., Hoffstein, S., Kaplan, H., Gennaro, D., Hirsch, J., and Fox, A. C., Clin. Res. **23,** 383A (1975).
16. Lefer, A. M., and Verrier, R. L., Clin. Pharmacol. Therap. **11,** 630 (1970).
17. Neely, J. R., Liebermeister, H., and Morgan, H. E., Amer. J. Physiol. **212,** 815 (1967).
18. Munck, A. and Wira, C. H., *in* "Advances in the Biosciences, Schering Workshop on Steroid Hormone Receptors" (G. Raspe, ed.), p. 301. Pergamon Press, New York (1970).
19. Baxter, J. D., and Tomkins, G. M., Proc. Nat. Acad. Sci. USA **68,** 932 (1971).
20. Travis, R. H., and Sayers, G., Endocrinology **62,** 816 (1958).

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