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Publication Date
2023-09-06

THE COMBINED STIMULATION OF THE RETICULOENDOTHELIAL SYSTEM BY ESTRADIOL AND ENDOTOXIN

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DISCLAIMER

ABSTRACT

Both endotoxin and estradiol increase the rate of removal of intravenously injected colloidal carbon. When endotoxin was administered to mice which had been pretreated with estradiol, a very marked acceleration of carbon clearance was attained which reached a value 10 times that of untreated animals. This increase in the rate of carbon clearance was also greater than the sum of the increases in the rates obtained with the two stimulants acting independently. Such a stimulation is consistent with the hypothesis that estradiol causes an increase in RE cell number and that a subsequent injection of endotoxin increases the activity of both the new cells and the old.

INTRODUCTION

For the past 15 years, the phagocytic function of the reticuloendothelial system has been studied quantitatively by a large number of workers using a variety of colloidal materials. It has been found that the rate of removal of intravenously injected colloids can be altered by the previous administration of many different agents, among these are bacterial endotoxins and estrogens.

With endotoxin, the usual response in mice seems to be a period of depression followed by marked stimulation (Biozzi, Benacerraf and Halpern, 1955; Benacerraf and Sebestyen, 1957; and Arredondo and Kampschmidt, 1963). In 1964, Freedman and Sultzer reported both depression and stimulation in phagocytic activity of mice in the first few hours following the injection of presumably identical endotxins prepared from the same strain of Salmonella typhosa. Arredondo and Kampschmidt (1963) and Kampschmidt, Upchurch and Park (1965) have found in rats a very marked stimulation at 2 hours, followed by a period of supra-normal activity. Filkins and Di Luzio (1969), in an extensive investigation, have also shown this very marked early stimulation and found that treating the animals with heparin before endotoxin prevents its manifestation in rats. While our experiments in mice, reported below, do not include studies on the first day, they show a marked stimulation at 48-72 hours.

With estradiol, the response is again one of stimulation. The observation by Nicol in 1935 that estrogenic hormones stimulated the activity of phagocytic cells in the endometrium led to the demonstration by Bilby and Nicol in 1958 that the rate of clearance of carbon from the circulating

blood was greatly increased by estrogenic steroids. In 1957, Heller, Meier, Zucker and Mast also showed that estrogens increased the rate of carbon phagocytosis. Kelly, Dobson, Finney and Hirsch (1960) and Kelly, Brown and Dobson (1962) have presented evidence that estradiol stimulation is accompanied by increased liver DNA synthesis and increased littoral cell number. In a more recent publication, Kelly and Dobson (1971) have presented further evidence indicating that estradiol stimulates the RE system largely by cell proliferation and that endotoxin does so largely by increasing the activity of existing cells. This suggested to us that perhaps stimulation by estradiol would produce more cells which might subsequently be activated by endotoxin to produce an even greater stimulation. While the experiments reported here do not establish the correctness of the proposed mechanism, they do establish that this regimen results in a large acceleration of the carbon clearance rate.

METHODS

Measurement of RES Activity

Phagocytic function was determined essentially by the method used by Benacerraf, Halpern, Biozzi and Benos (1954). Six mg. per mouse of colloidal carbon[1] were injected intravenously. Blood was collected from the tip of the tail at appropriate time intervals. The concentration of the carbon was measured spectrophotometrically and plotted on semilog paper as a function of time. The fractional disappearance rate constant, k, defined by:

$$C = C_o e^{-kt}$$

has been used as a quantitative measure of phagocytic activity of the RE system[2].

The initial carbon phagocytic rate used in Fig. 4 was calculated by simply multiplying the fractional disappearance rate constant, k, by the amount of carbon injected.

Swiss male mice, 20-30 grams, were used in all experiments.

RES Stimulation

Estradiol: In all experiments involving estradiol, 1.0 mg. of estradiol (Mann Research Laboratories, General Biochemicals) in 0.2 ml sesame oil was injected subcutaneously. (The subcutaneous injection of sesame oil was found to have no effect on carbon clearance.)

Endotoxin: E. coli endotoxin (Difco lipopolysaccharide 026:B6) in 0.1 ml saline was injected intravenously. All experiments, except the dose response experiment, were done with Preparation #107637. The supply of this preparation was exhausted, and the dose response measurements were

made with Preparation #117779, a somewhat more refined product.

RESULTS

Estradiol

Estradiol has been used as a stimulator of the reticuloendothelial system in this laboratory for a number of years. The data points shown in Fig. 1 are means from these many experiments, involving a total of 373 mice, in which carbon clearances were measured at various times after estradiol administration (1 mg per mouse). Each experimental point is an average of 3 to 12 animals. To avoid confusion, standard errors of the mean for the highest and lowest points at each time interval are the only ones shown. An unexplained result is that the difference between the highest and lowest points at 3 days and at 4 days is statistically significant. Despite this anomalous variability in the magnitude of the response, a gradual increase in phagocytic activity as indicated by the carbon clearance rate constant, k, always occurred with time. This increase reached its peak at 3 to 5 days and then seemed to fall off rather sharply.

Endotoxin

In order likewise to establish a time function for endotoxin activation, a single injection of 0.1 mg *E. coli* endotoxin was given, and carbon clearances were measured 2, 3, 5, 6, and 10 days later. Fig. 2 shows the carbon disappearance constants of the stimulated mice plotted as a function of time. The very marked stimulation, nearly 2½ fold, seen at three days falls off so that by 5 days the activity is only 50% above normal.

While doses of endotoxin from 0.005 mg to 0.025 mg resulted in very marked increases in the rate of carbon clearance, doses of endotoxin ranging

from 0.025 to 0.1 mg seemed to produce relatively small additional changes in the stimulatory effect. The data are presented in Fig. 3 with the phagocytic activity of 2-day stimulated animals plotted as a function of the endotoxin dose administered. The data actually fit a log-dose response relationship reasonable well. Higher doses of endotoxin were not measured because the 0.1 mg dose of this lot of endotoxin tended to make the animals sick and frequently caused deaths. In the additivity experiments reported below, which in actuality were performed before the dose response experiment, a less refined lot of endotoxin was used. With 0.1 mg of this endotoxin, no lethality was observed. In fact, it was the observation that the 0.1 mg of the new endotoxin showed considerable toxicity that led to the dose response experiments.

Estradiol and Endotoxin in Combination

The combined effect of endotoxin and estradiol were studied in two separate, nearly identical, experiments. In one, estradiol was allowed to act for 2 days before endotoxin was given. The disappearance rates were measured 2 days after the endotoxin (4 days after estradiol). In the second experiment, estradiol was allowed to act for 3 days before the endotoxin was given, and carbon clearances were again measured 2 days post endotoxin (5 days post estradiol). These data are shown in Fig. 4.

Both estradiol and endotoxin separately showed stimulation of the reticuloendothelial system, but the combined effect of endotoxin and estradiol produced a marked increase in the rate of carbon clearance, elevating the rate constant by more than a factor of 10 above normal.

No increase in endotoxin lethality was observed in the estradiol stimulated mice.

DISCUSSION

A gradual, though highly variable, increase in phagocytic activity with respect to time following estradiol administration is shown in Fig. 1. This increase correlates with cell population studies published previously (Kelly and Dobson, 1971). We have no explanation for the very marked variability in the magnitude of the estradiol response seen in Fig. 1, nor can we explain the very rapid fall-off in activity which seems to occur at 6 days. However, Ware and Nicol (1960) found a similar sharp decrease four days following the cessation of multiple injections of diethylstilbestrol. It should be pointed out that the increased rate of carbon clearance produced by estradiol probably is due in part to factors other than cell population changes.

Fig. 2 shows that endotoxin stimulation is very nearly as great at 2 days as at 3 days. It seemed efficacious, therefore, to administer the endotoxin 2 to 3 days after the estradiol so that carbon clearances could be measured close to the peak of each type of stimulation.

Fig. 4 shows the combined effect of estradiol and endotoxin administered sequentially. Since the plateauing of the response shown in Fig. 3 suggests that increasing the endotoxin dose level would not result in pronounced additional increases in phagocytic activity at higher nearly lethal levels, the marked effect of the combined administration in Fig. 4 suggests again that estradiol and endotoxin stimulation are different. The observed fact is that the fractional disappearance rate constant of the doubly stimulated animals exceeded 10 times that of the untreated controls. Such a marked stimulation, one greater than additive, can be explained by the

hypothesis that while estradiol stimulates existing cells to some extent, it greatly increases the number of cells; and endotoxin, acting on this expanded population, subsequently activates these new cells as well as the old ones.

The combined stimulation of estradiol and endotoxin produced individuals with clearances as high as 43% per minute and half-times of carbon disappearance of 1.6 minutes. In this range, blood flow begins to be an important factor in limiting the rate of clearance (Dobson, 1957; Fred, Harris, Parker and Shore, 1967). Half-times of this magnitude correspond to single pass extractions of 20-30%. It has been shown with chromic phosphate, which is essentially completely cleared from the blood in a single passage through the liver, that normal mice have half-times of 30-40 seconds (Dobson, Finkelstein, Finney and Kelly, 1966). Such half-times for colloids localizing in the liver and spleen are governed by liver blood flow (Dobson and Jones, 1952) and cannot be expected to accurately indicate degrees of stimulation of the RES. Thus, even if the RE cells were stimulated to infinte activity, any colloid that is completely cleared in a single pass can be cleared no faster. The rate of delivery to the cells limits the rate of removal.

In the additivity experiments described in this paper, the stimulation of activity to ten times normal is probably close to the region beyond which blood flow limitations must be considered.

Benacerraf, Thorbecke and Jacoby (1959) using zymosan, and Crafton and Di Luzio (1969) using glucan to stimulate the RES to hyperactivity, greatly increased the sensitivity of mice and rats to endotoxin. Both stimulating agents reduced the LD_{50} by a factor of 40 to 100. Clearly, no such increase

in endotoxin lethality was produced by estradiol stimulation of the RES in the present experiment. Similarly, Trejo, Loose and Di Luzio (1972) reported that stimulation of the RES by the synthetic estrogen, diethylstilbestrol, was not accompanied by increased sensitivity to endotoxin.

In conclusion, large doses of estradiol and endotoxin can be combined in sequential administrations to produce very marked stimulation of the reticuloendothelial system.

REFERENCES

Arredondo, M.I., and Kampschmidt, R.F. (1963). Effect of endotoxins on phagocytic activity of the reticuloendothelial system of the rat. Proc. Soc. Exptl. Biol. Med. 112, 78-81.

Benacerraf, B., Halpern, B.N., Biozzi, G., and Benos, S.A. (1954). Quantitative study of the granulopectic activity of the reticulo-endothelial system. III: The effect of cortisone and nitrogen mustard on the regenerative capacity of the R.E.S. after saturation with carbon. Brit. J. Exptl. Pathol. 35, 97-106.

Benacerraf, B. and Sebestyen, M.M. (1957). Effect of bacterial endotoxins on the reticuloendothelial system. Fed. Proc. 16, 860-867.

Benacerraf, B., Thorbecke, G.J., and Jacoby, D. (1959). Effect of zymosan on endotoxin toxicity in mice. Proc. Soc. Exptl. Biol. Med. 100, 796-799.

Bilbey, D.J.L., and Nicol, T. (1958). Effect of various natural steroids on the phagocytic activity of the reticuloendothelial system. Nature 182, 674.

Biozzi, G., Benacerraf, B., and Halpern, B.N. (1955). The effect of Salm. typhi and its endotoxin on the phagocytic activity of the reticulo-endothelial system in mace. Brit. J. Exptl. Pathol. 36, 226-235.

Crafton, C.G., and Di Luzio, N.R. (1969). Relationship of reticuloendothelial functional activity to endotoxin lethality. Am. J. Physiol. 217, 736-742.

Dobson, E.L. (1957). Factors controlling phagocytosis. In "The Physiopathology of the Reticulo_endothelial System," (B.N. Halpern, B. Benacerraf and J.F. Delafresnaye, eds.), 80-114. Blackwell.

Dobson, E.L., Finkelstein, L.J., Finney, C.R., and Kelly, L.S. (1966). Particulate chromic phosphate: Colloid characteristics suitable for measurement of liver circulation. In "Radioactive Pharmaceuticals" (G.A. Andrews, R.M. Kniseley and H.N. Wagner, Jr., eds.), pp 477-502. U.S. Atomic Energy Commission, Div. of Technical Information.

Dobson, E.L., and Jones, H.B. (1952). The behavior of intravenously injected particulate material: Its rate of disappearance from the blood stream as a measure of liver blood flow. Acta med. scandinav. 144: Suppl. 273.

Dobson, E.L., Kelly, L.S., and Finney, C.R. (1967). Kinetics of the phagocytosis of repeated injections of colloidal carbon: Blockade, a latent period or stimulation? A question of timing and dose. In "Advances in Experimental Medicine and Biology" (N.R. Di Luzio and E. Paoletti, eds.), Vol. 1, pp 63-73. Plenum Press, New York.

Filkins, J.P., and Di Luzio, N.R. (1969). Influence of opsonins and heparin on intravascular carbon clearances after endotoxin. RES, J. Reticuloendothelial Soc. 6, 287-299.

Fred, R.K., Harris, J.G., Parker, H.G. and Shore, M.L. (1967). A mathematical model of RES phagocytic function. RES, J. Reticuloendothelial Soc. 4, 524-542.

Freedman, H.H., and Sultzer, B.M. (1964). Opposite changes in phagocytic activity induced by two endotoxins. RES, J. Reticuloendothelial Soc. 1, 216-223.

Heller, J.H., Meier, R.M., Zucker, R., and Mast, G.W. (1957). The effect of natural and synthetic estrogens on reticuloendothelial system function. Endocrinol. 61, 235-241.

Kampschmidt, R.F., Upchurch, H.F., and Park, A. (1965). Further studies on carbon clearance after endotoxin in the rat. RES, J. Reticuloendothelial Soc. 2, 256-262.

Kelly, L.S., Brown, B.A., and Dobson, E.L. (1962). Cell division and phagocytic activity in liver reticulo-endothelial cells. Proc. Soc. Exptl. Biol. Med. 110, 555-559.

Kelly, L.S. and Dobson, E.L. (1971). Evidence concerning the origin of liver macrophages. Brit. J. Exptl. Path. 52, 88-99.

Kelly, L.S., Dobson, E.L., Finney, C.R., and Hirsch, J.D. (1960). Proliferation of the reticuloendothelial system in the liver. Am. J. Physiol. 198, 1134-1138.

Nicol, T. (1935). The female reproductive system in the guinea-pig: Intravitam staining; fat production; influence of hormones. Trans. Roy. Soc. Edin. 58, 449-486.

Parker, H.G., and Finney, C.R. (1960). Latent period in the induction of reticuloendothelial blockade. Am. J. Physiol. 198, 916-920.

Trejo, R.A., Loose, L.D., and Di Luzio, N.R. (1972). Influence of diethylstilbestrol (DES) on reticuloendothelial function, tissue distribution, and detoxication of S. enteritidis endotoxin. RES, J. Reticuloendothelial Soc. 11, 88-97.

Ware, C.C., and Nicol, T. (1960). Duration of stimulation of phagocytic activity of the reticulo-endothelial system after cessation of treatment with diethylstilboestrol. Nature 186, 974-975.

Footnotes

(1) Gunther Wagner, Hanover, Germany, Suspension no. c11/1431a, prepared as described by Parker and Finney (1960).

(2) The approximation of the disappearance curve to an exponential function and the relationship of the disappearance constant to the "phagocytic index" is discussed by Dobson, Kelly and Finney (1967).

Legends for Figures

Fig. 1 The carbon clearance rate constant, k, as a function of time in days after administration to mice of 1 mg of estradiol. The data points shown are individual means from many separate experiments. Standard errors are shown only for the highest and lowest means for any one time. Values to the left of the zero line represent uninjected controls.

Fig. 2 The carbon clearance rate constant, k, as a function of time in days after administration to mice of 0.1 mg of endotoxin. Uninjected controls are plotted at zero time.

Fig. 3 The carbon clearance rate constant, k, as a function of endotoxin dose. All carbon clearance measurements were made at 2 days following the intravenous injection of endotoxin. Uninjected controls are plotted at zero dose.

Fig. 4 The carbon disappearance rate in mg per minute is shown for normal mice, estradiol stimulated mice, endotoxin stimulated mice, and for mice stimulated first with estradiol and subsequently with endotoxin. Experiment A and Experiment B are alike, except that in Experiment B estradiol was allowed to act one day longer before endotoxin was given.