Interactions of Indomethacin with Lysosomes and Proteins

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Abstract □ The stabilizing action of indomethacin on lysosomes and protein *in vitro* was studied. The magnitude of the stabilizing action on lysosomes was greater than that of aspirin or oxyphenbutazone, similar to that of phenylbutazone, but less than that of prednisolone. The stabilizing action of indomethacin on lysosomes possibly may contribute to its anti-inflammatory properties.

Keyphrases □ Indomethacin—effect on lysosome and protein stability *in vitro*, compared to other drugs □ Lysosomes—stability, effect of indomethacin *in vitro*, compared to other drugs □ Protein—stability, egg albumin, effect of indomethacin *in vitro*, compared to other drugs □ Anti-inflammatory agents—indomethacin, effect on lysosome and protein stability *in vitro*, compared to other drugs □ Stability—lysosome and protein *in vitro*, effect of indomethacin, compared to other drugs

It was reported (1) that indomethacin has little action on lysosomes but stabilizes canine and human erythrocytes from heat-induced and hypotonic rupture (2). It was suggested (2) that the stabilizing action of the drug was due to its stabilizing action on membrane proteins and that erythrocyte and lysosomal membranes have certain common properties.

Variations in experimental procedures can lead to different results in lysosome stabilization experiments (3). This study compared the action of indomethacin on lysosomes with that of other drugs under identical conditions and over a wide concentration range. The action of indomethacin on protein stability also was studied.

EXPERIMENTAL

Action of Indomethacin on Lysosomes—Lysosomes were isolated, as previously described (4), from freshly excised rat liver homogenates in 0.05 *M* tromethamine–acetate-buffered sucrose (0.25 *M*) (pH 7.4). The homogenate was subjected to differential centrifugation[1] at 4° and the lysosomes were isolated in the fraction sedimenting at 10,000×*g* after 20 min of centrifugation. Then the lysosomes were suspended in tromethamine–acetate-buffered isotonic sucrose (pH 7.4) to a final protein concentration of 5 mg/ml. The drugs were dissolved in dimethyl sulfoxide, and portions (0.1 ml) were added to 5 ml of the lysosome suspension (5 mg of protein/ml) in tromethamine–acetate-buffered isotonic sucrose (pH 7.4).

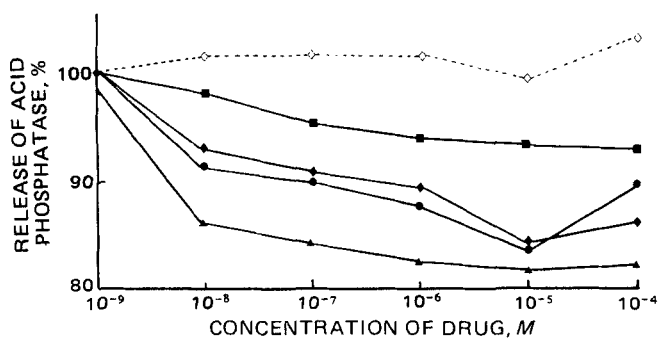

Figure 1—*Action of indomethacin and other drugs on lysosomes. Values below 100% (control values) represent a stabilizing action by the drug. Values greater than 100% represent a lytic action by the drug. Each point represents the mean of four determinations for each concentration. Key: ◊, aspirin; ■, oxyphenbutazone; ●, phenylbutazone; ♦, indomethacin; and ▲, prednisolone.*

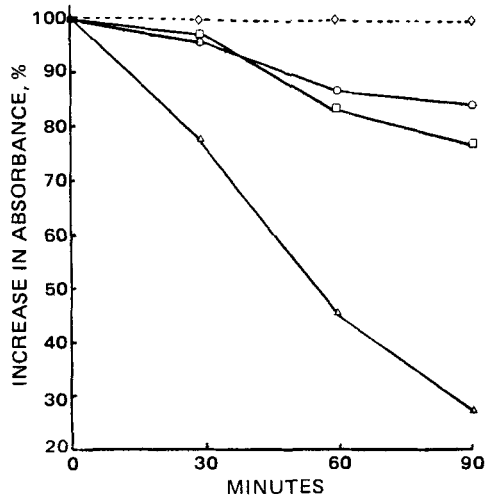

Figure 2—*Effect of indomethacin on protein stabilization. The control values represent a mean of nine determinations, and the test results represent a mean of three determinations for each concentration. The control values were adjusted to 100% at each reading, and the experimental values were calculated as a percent of that figure. Values below 100% represent a stabilizing action by the drug on the protein. Key: □, 10^{-2} M; ○, 10^{-7} M; △, 10^{-5} M; and ◊, controls.*

The suspensions were incubated for 90 min at 37° in a shaking reaction incubator and then centrifuged at 20,000×*g* for 20 min. The acid phosphatase activity was determined by incubating 0.1 ml of the supernate with 0.05 ml of *p*-nitrophenyl phosphate[2] (0.015 *M*) and 0.5 ml of 0.09 *M* citrate buffer (pH 4.8) for 30 min at 37°. The reaction was stopped by the addition of 0.1 *M* NaOH, and the liberated *p*-nitrophenol was determined at 410 nm. Drugs were omitted from the controls.

Action of Indomethacin on Proteins—Portions (0.1 ml) of the drug in dimethyl sulfoxide were incubated with 5 ml of egg albumin solution (pH 7.4) at 65° in a water bath, and the absorbance was measured periodically at 420 nm (4). The drug was omitted from the controls.

RESULTS

Action of Indomethacin on Lysosomes—The results (Fig. 1) show that indomethacin stabilized the lysosomes over a wide concentration range. The effect was greater than that of aspirin (which did not stabilize the lysosomes) and oxyphenbutazone, similar to that of phenylbutazone, but less than that of prednisolone.

Action of Indomethacin on Proteins—Indomethacin stabilized the protein against thermal denaturation, with maximum values at 10^{-5} *M* (Fig. 2). The fall off in stabilization at 10^{-2} *M* may be a consequence of solubility of the drug–protein complexes at this high concentration since immediate precipitation of the complex occurred at concentrations above this value ($10^{-1.3}$ and 10^{-1} *M*). This result is in agreement with previous findings that many nonsteroidal drugs stabilize proteins (5, 6).

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Effect of Pentazocine on Pressor Responses of Epinephrine and Levarterenol

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Abstract □ In rats anesthetized with pentobarbital, pentazocine potentiated the pressor response of two exogenous amines, epinephrine and levarterenol. Although the mechanism for the pentazocine-induced potentiation of the pressor amines has not been proven, it is speculated that pentazocine may increase the blood pressure response of certain amines by interacting with the sympathetic nervous system.

Keyphrases □ Pentazocine—effect on pressor responses of epinephrine and levarterenol, rats □ Epinephrine—pressor responses, effect of pentazocine, rats □ Levarterenol—pressor responses, effect of pentazocine, rats □ Pressor responses—epinephrine and levarterenol, effect of pentazocine, rats □ Analgesics—pentazocine, effect on pressor responses of epinephrine and levarterenol, rats □ Adrenergic agents—epinephrine and levarterenol, pressor responses, effect of pentazocine, rats

Pentazocine, a weak narcotic antagonist of the benzomorphan series, was first synthesized by Archer *et al.* (1). It is an effective analgesic in humans (2) with a low incidence of adverse effects (3).

Whereas the cardiovascular effects of morphine are hypotension and bradycardia, pentazocine produces a rise in systemic arterial blood pressure and heart rate (2). Therefore, Hobler *et al.* (4) suggested that pentazocine might be a useful analgesic and less dangerous than morphine in hypotensive patients.

The present study concerned the possibility that pentazocine might increase blood pressure and heart rate by altering the responses to transmitter amines. This paper reports the effects of pentazocine on the pressor responses to exogenous epinephrine (I) and levarterenol (II).

EXPERIMENTAL

Male Sprague–Dawley rats were anesthetized with pentobarbital sodium, 40 mg/kg ip, combined with atropine sulfate, 1 mg/kg. Animals were artificially respired throughout the experiment with a rodent respirator[1]. The right carotid or femoral artery was cannulated with polyethylene tubing filled with heparin in saline to facilitate pressure measurements using a pressure transducer[2]. The right femoral vein was cannulated for intravenous injections of drugs.

Pressure changes were analyzed by planimetric measurement of the area, in square centimeters, under the dose–mean blood pressure response curve and by measurement of the time, in seconds, for the mean blood pressure response to return half-way to the control value. Statistical analysis was performed by using the analysis of variance combined with Duncan's new multiple range test (5). Levarterenol bitartrate[3] and epinephrine bitartrate[3] solutions were prepared from stock solutions on the day of use. Pentazocine[4] was dissolved in acidic aqueous solutions prior to use. All doses are reported as the salt.

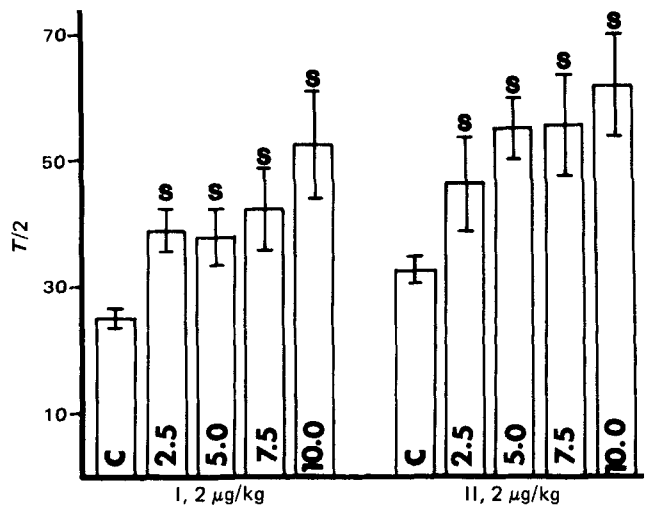

Figure 1—*Effect of intravenous pentazocine (milligrams per kilogram shown in the bars) on the duration (T/2, in seconds) of the pressor responses to I and II. All doses of pentazocine significantly (S) potentiated the duration of the pressor responses to I and II when compared to the control (C) at* $p < 0.05$.

RESULTS AND DISCUSSION

Epinephrine, 2 μg/kg, and levarterenol, 2 μg/kg, were given intravenously to rats to determine control blood pressure responses. Pentazocine was given in 2.5-mg/kg increments intravenously until a total dose of 10 mg/kg had been administered. The intravenous administration of pentazocine routinely depressed the blood pressure, but the depression was transient and the blood pressure was allowed to return to control levels before the agonists were given.

After each dose of pentazocine, the agonists were again administered and the responses were measured and compared to control responses. All doses of pentazocine significantly increased the duration of the pressor responses to epinephrine and levarterenol (Fig. 1), while the area under the dose–response curve of these two amines was significantly increased only after a 10-mg/kg dose (Fig. 2).

Since these experiments involved a long period and several injections per animal, a study was conducted to evaluate the possibility that the responses to epinephrine and levarterenol might be increased by some sort of autopotentiation instead of an action by pentazocine. Blood pressure responses to four doses of epinephrine were obtained several

[1] Harvard Apparatus Co. model 680.
[2] Statham P23AC.
[3] Sigma Chemical Co., St. Louis, Mo.
[4] Talwin, Sterling-Winthrop Research Institute.