Comparison of the Protective Properties of Soterenol and Isoproterenol against Endotoxin Lethality (34408)

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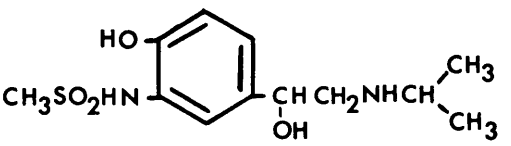
Soterenol (MJ 1992), a methanesulfonamidophenethanolamine, synthesized by Larsen *et al.* (5) is structurally related to isoproterenol (Fig. 1). Previously reported pharmacological evaluations indicate that soterenol is predominantly a β-adrenergic stimulating agent that possesses, to a lesser extent, α-adrenergic stimulating action (2). The bronchodilating properties of the two drugs compare favorably; however, the undesirable cardiac side-effects are reported to be less with soterenol (2).

This report compares the two drugs in their ability to reduce endotoxin lethality in chick embryos and mice.

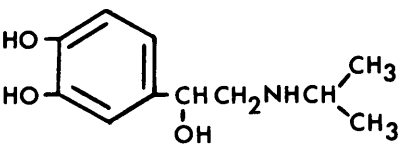
Materials and Methods. Endotoxin. Commerically prepared endotoxin from *Escherichia coli* 0111:B4 (Difco) was used throughout this study. An intravenous dose of 25 mg/kg to mice resulted in 75–80% deaths within 72 hr. An 86% mortality was obtained in the chick embryo system with an intravenous dose of 0.01 μg/egg. All solutions were made in sterile 0.15 *M* NaCl.

Drugs. Isoproterenol (Isuprel) was obtained from Winthrop Laboratories and soterenol was provided by Mead Johnson. Each compound was used as its hydrochloride salt, and all solutions were prepared in sterile 0.15 *M* NaCl.

Mouse lethality test. Male albino mice (Laboratory Supply, Indianapolis) were given tetracycline in their drinking water for 2 days followed by at least 2 days of untreated water prior to being placed on test (8). This procedure resulted in a more consistent mortality of the endotoxin control mice. Food and water were provided *ad libitum*. Mice



Soterenol



Isoproterenol

FIG. 1. Chemical structures.

were housed five animals per cage in a constant environment of 70–75°F and 50% relative humidity. The drugs were injected intravenously, followed approximately 1 hr later by intravenous administration of endotoxin. The animals were observed for 72 hr and the survival rate was determined.

Chick embryo model. Fertile chicken eggs (white leghorn) were obtained from a commercial hatchery. After 10-days incubation in a Jamesway single stage incubator, the embryos were candled, prepared, and injected according to the method of Gruninger and Spink (3).

Varying concentrations of drug and endotoxin were mixed in equal volumes and 0.1 ml of the mixture was immediately injected intravenously. Endotoxin concentration of 0.01 μg/egg was kept constant. After 24-hr incubation the treated eggs were candled and

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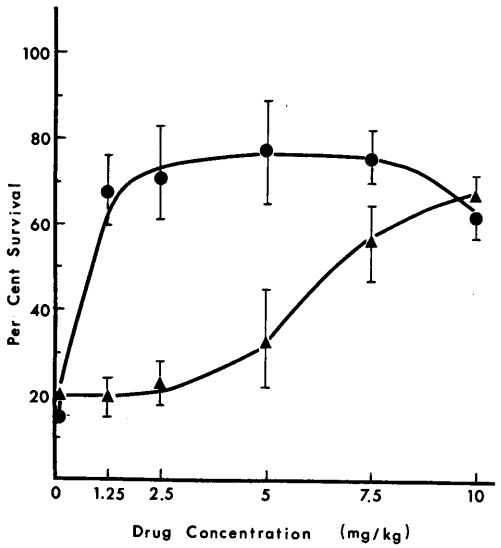

FIG. 2. Dose–response curve of soterenol (●) and isoproterenol (▲) modifying the lethal effects of 25 mg/kg of endotoxin in the mouse model. Points on curves represent the mean response ± SE of 4–9 groups of 9–10 mice each.

the viability was determined. A gross examination of all dead embryos was made. Embryos not exhibiting the characteristic hemorrhage caused by endotoxin were not included in the calculation of survival rates.

Results. Mouse lethality test. Results from the mouse lethality test are graphically illustrated in Fig. 2. Soterenol was significantly more protective than isoproterenol at concentrations below 7.5 mg/kg as determined by the *t* test. The overall difference is significant at the 0.01 level as determined by the chi-square test. This difference in potency is shown clearly at the 1.25–5.0 mg/kg levels. At the lowest level, soterenol protected 67.5% of the mice, while isoproterenol protected only 19%. At 7.5 mg/kg, the difference between the two drugs was not significant. Above 10 mg/kg, toxic effects were observed with both drugs.

Chick embryo model. Results obtained with the embryo model support those observed with mice (Fig. 3). A dose of 0.012 μg/egg resulted in 42.9% survival with soterenol as compared to only 16.1% survival with isoproterenol, and this difference was significant at the 0.001 level (*t* test). The overall difference between the two drugs is significant at the 0.05 level as determined by the chi-square test. This difference is not as great, nor does it exist for as many dose levels, as in the mouse model (Figs. 2, 3). Both drugs were well tolerated by the embryos. Using doses of 0.3 μg/egg, 100% survival of the embryos was observed with both soterenol and isoproterenol.

Discussion. The treatment of endotoxin shock remains a major medical problem. It is reported that endotoxin shock appears in 20–30% of patients with gram-negative bacteremia, and the mortality rate varies from 30 to 80% (6).

Clinically, to combat the vascular symptoms of shock, three main types of drugs are used: steroids; α-adrenergic blocking agents; and β-adrenergic stimulating agents, such as isoproterenol.

The use of isoproterenol in the treatment of shock is well documented in animals and man. Gruninger and Spink (3) protected chick embryos with as little as 0.1 μg of isoproterenol. Starzecki *et al.* (9) reported a significant increase in survival of endotoxin-shocked dogs treated with isoproterenol. Twelve human patients with endotoxin shock were treated with isoproterenol by Kardos (4). Eight of 12 survived, three of the four deaths were attributed to underlying causes. Isoproterenol has also been included in the

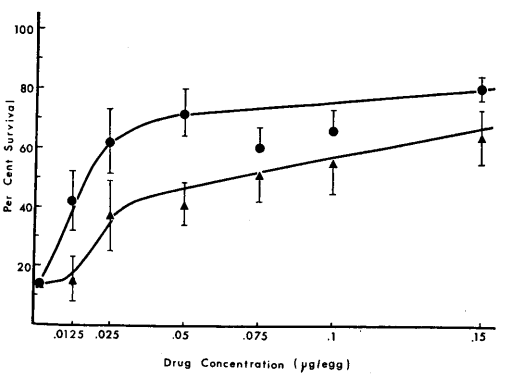

FIG. 3. Dose response curve of soterenol (●) and isoproterenol (▲) modifying the lethal effects of 0.01 μg/egg of endotoxin in the chick embryo model. Points on curves represent the mean response ± SE of 38–71 embryos/group (composite of 4–6 expts.).

treatment of endotoxin shock by other investigators (1, 6).

The observations reported here indicate that the similarities between isoproterenol and soterenol can be extended to include their ability to protect animals from the effects of endotoxin. The findings of Gruninger and Spink (3) were confirmed regarding the levels of isoproterenol needed to protect the chick embryo from the lethal effects of endotoxin. It was also shown that soterenol provided greater protection than observed with isoproterenol when compared at lower concentrations of drug.

The ability of β-adrenergic stimulators to partially protect animals from the lethal effects of endotoxin can be explained in several ways: (i) the increased heart rate and relaxation of the peripheral blood vessels promote a more rapid clearing and detoxification of the endotoxin; (ii) a direct interaction between the endotoxin and the drug results in increased detoxification and/or decreased toxicity; and (iii) the β-adrenergic stimulant and the endotoxin might compete for β-adrenergic receptor sites. The present data do not exclude any of these possibilities. However, preliminary data obtained in this laboratory indicate that pretreatment of mice with a β-adrenergic blocker (Sotalol, MJ 1999) potentiates the lethal effects of endotoxin, lending support to the third possibility. The recent report by Pieroni and Levine (7) to the effect that insulin enhances endotoxin lethality suggests that the blood glucose level may play a pivotal role in determining susceptibility to endotoxin.

Summary. The ability of soterenol and isoproterenol to reduce endotoxin lethality in chick embryos and mice was compared. Soterenol was significantly more potent than isoproterenol at lower concentrations in both models. At higher concentrations (10 mg/kg in mice of 0.075 μg/egg) the difference between the drugs was not significant. The role of β-adrenergic stimulators in combating endotoxin lethality was discussed.

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1. Blair, E., Wise, A., and Mackey, A. G., J. Am. Med. Assoc. **207**, 333 (1969).

2. Dungan, K. W., Cho, Y. W., Gomoll, A. W., Aviado, D. M., and Lish, P. M., J. Pharmacol. Exptl. Therap. **104**, 290 (1968).

3. Gruninger, R. P. and Spink, W. W., Proc. Soc. Exptl. Biol. Med. **124**, 1277 (1967).

4. Kardos, G. G., New Engl. J. Med. **274**, 868 (1966).

5. Larsen, A. A., Gould, W. A., Roth, H. R., Comer, W. T., Uloth, R. H., Dungan, K. W., and Lish, P. M., J. Med. Chem. **1**, 462 (1967).

6. Pacheco, J. C. and Martin, W. J., Minn. Med. **51**, 493 (1968).

7. Pieroni, R. E. and Levine, L., Experientia **25**, 507 (1969).

8. Snyder, I. S., Agarwal, M. K., and Berry, L. J., J. Bacteriol. **94**, 1817 (1967).

9. Starzecki, B., Reddin, J. L., and Spink, W. W. Ann. Surg. **167**, 35 (1968).

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