Ammonolyzed Epinephrine Conjugates and Their Pressor Action in Dogs

Richard G. Roberts and Herman J. Horvitz

Chicago Medical School, Chicago, Illinois

When epinephrine is treated with anhydrous liquid ammonia an ammonolyzed derivative is formed (1). This new product on intravenous injection causes a more prolonged (50% longer) elevation of blood pressure in dogs than epinephrine, although the extent to which the pressure is elevated is approximately the same. Because this is obviously a desirable pharmacological feature of the new product, an attempt has been made to augment this feature by conjugating one of the normal constituents of blood plasma with epinephrine.

The substances chosen to be conjugated with epinephrine were glycine, tyrosine, glutamic acid, urea, lactic acid, dextrose, cebione and cholesterol. Liquid ammonia was used as a solvent and dispersing medium because the changes wrought by conjugation in liquid ammonia are generally more marked than those obtained by ammonolysis alone. For example, by conjugating glycine with hematin in liquid ammonia a compound is formed which retains the properties of the original pigment and yet is soluble in water at pH 7.0 to 7.4.

Methods: The method used was essentially the same as that described in the first paper (1). The liquid ammonia was dried over sodium by the method of Fernelius and Johnson (2). The combined sample (epinephrine plus the substance to be added to it) used for a single reaction was usually less than 0.5 gm. The reaction was carried out in a heavy walled, transparent Dewar flask which was attached to a mercury seal so that the excess liquid ammonia could boil off without moisture being admitted to the epinephrine. This required about 24 hours. 200 cc. of liquid ammonia were used. The Dewar flask was then attached to a vacuum pump until the odor of ammonia was removed. The flask was not heated. The dry powder was then ready for use, and was made up to a concentration of one mgm. per cc. in terms of its epinephrine content. Distilled water and ethylene glycol were the only vehicles used. Glycerol was abandoned as a vehicle in favor of ethylene glycol due to the lower viscosity of the latter.

Dogs weighing about 12 kgm. were used to assay the new compounds. The injections were made into the femoral vein, and the carotid blood pressure was measured by the customary method. Ether and sodium phenobarbital were the anaesthetics used. At least twenty minutes were allowed to elapse between injections.

Results: It was found that epinephrine dried to constant weight in a vacuum desiccator over sulfuric acid is essentially insoluble in liquid ammonia in a concentration of 25 mgm. of epinephrine to 200 cc. of liquid ammonia. The powder swells slightly and becomes darker, and the liquid ammonia continues to boil faster for thirty minutes or more indicating some kind of an exothermic reaction between the epinephrine and the liquid ammonia.

Glycine is exceedingly soluble in liquid ammonia and when epinephrine is added to such a solution of glycine in ratios of 0.5 to 10 moles of glycine to one of epinephrine, the epinephrine is brought into solution or is very finely dispersed, and the clear solution becomes yellowish. A light flocculent precipitate then forms quickly, but does not begin to settle for an hour or more, although epinephrine itself settles immediately. The new product in concentrations of 1:1000 is not entirely soluble in either water or ethylene glycol, but it can be injected in the form of a finely dispersed sol. Water

can be used as a vehicle, but the injection must be made at once as the desiccator. Observing the above precautions, the glycine and epinephrine of obtained. In ethylene glycol, however, the derivative remains active for weeks, even in an open beaker. The activity is retained in glycerol also. The dry powdered derivative retains its activity in well stoppered bottles or sealed ampoules, but loses it when kept over sulfuric acid in a vacuum desiccator. Observing the above precautions, the glycine and epinephrine of equal molar proportions gives when injected intravenously a blood pressure

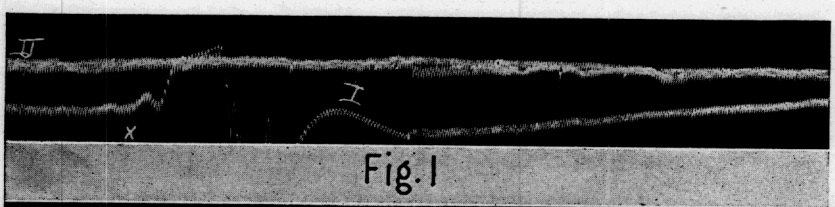

Fig. 1

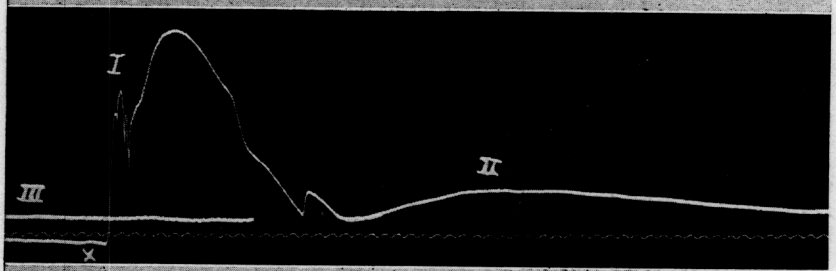

Fig. 2

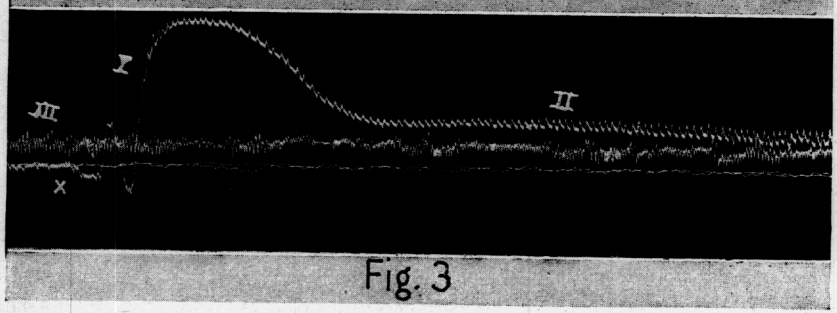

Fig. 3

EXPLANATION

Fig. 1. X = Base line and site of injection. I = Rise from below base line following primary excursion and continuation upward. II = Elevation reached by rise I, and maintained for one hour when drum was stopped. Time of drum for one rotation was twenty-five minutes. Ether anaesthesia was used. Dose used was 0.25 ccc. of 1:1000 epinephrine equivalent for a 1:1 molar conjugate of epinephrine and glycine. The vehicle was glycerol.

Fig. 2. X = Base line and site of injection. I is the primary excursion. II marks the prolongation of the secondary which did not fall below the base line. III is a continuation of rise II after twenty-five minutes. Ether anaesthesia was used. Dose used was 0.25 cc. of 1:1000 epinephrine equivalent for a 1:1 molar conjugate of epinephrine and dextrose. The vehicle was ethylene glycol.

Fig. 3. X = Base line and site of injection. I is the primary excursion. II marks the prolongation of the secondary which did not fall below the base line. III is a continuation of rise II after twenty-five minutes. Sodium phenobarbitol anaesthesia was used. Dose used was 0.25 cc. of 1:1000 epinephrine equivalent for a 1:1 molar conjugate of epinephrine and cholesterol. The vehicle was ethylene glycol.

curve that in a general way resembles the curve of the epinephrine control, but the peak of the primary curve is less sharp and the secondary portion of the curve is much prolonged. The secondary portion of the curve may return to the control blood pressure level or even pass below it within five to ten minutes as with epinephrine, but on rising again it does not remain on the base line, but continues to rise above it reaching an elevation of ten to fifty per cent of that of the initial rise, and maintaining such an elevation from one to three hours. The product resulting from the mole for mole proportion of epinephrine and glycine produced a more prolonged rise in pressure than the others; the other glycine derivatives produced a more prolonged blood pressure rise than epinephrine. (See Fig. I).

Other amino acids, such as tyrosine and glutamic acid, react with epinephrine in liquid ammonia to form derivatives that give a sustained rise in blood pressure. However, the secondary sustained rise in pressure is in general not as great as with the one to one epinephrine and glycine. Aihara and Ito claim potentiated adrenaline constriction of the perfused rabbit ear by the addition of aspartic acid or asparagine to the adrenaline perfusate. (4) We have used such mixtures for our control injections, and we have found the prolongation to be very slight or negligible.

Dextrose and cholesterol both form derivatives with epinephrine and these give a prolonged pressor action, although their mode of formation is somewhat different. Cholesterol and epinephrine are slightly soluble and form a derivative that is slightly soluble in liquid ammonia. Dextrose is exothermically very soluble, and forms a soluble derivative with epinephrine. Both of these derivatives produce a prolonged rise in blood pressure, and the secondary portion of the curve does not fall below the control blood pressure. (See Figs. 2 and 3).

Cebione or vitamin C is exothermically soluble in liquid ammonia, and forms a derivative with epinephrine that is soluble. This derivative does not have a prolonged pressor action, but it possesses a property that seems to be unique among the compounds examined. When left in an open beaker dispersed in ethylene glycol, it does not turn pink or red as do all of the other derivatives mentioned, but retains its original faint lemon yellow color for weeks; its pressor activity remains undiminished after a month. When left in water it turned red as did the other compounds, which is probably due to the liberation of epinephrine by hydrolysis.

The ease with which our ammonolyzed epinephrine conjugates hydrolyze might indicate that they are coordination products formed through the latent valences or hydrogen bridges (3) of the nitrogen in the liquid ammonia. They are quite stable when sealed or dispersed in ethylene glycol, however, and their long sustained pressor action indicates that they are not easily broken down in the blood. Our products have shown no evidence of being toxic, and due to their prolonged action over that of epinephrine they should prove to have a therapeutic value as hemostatics and as drugs for the treatment of hay fever and asthma.

SUMMARY

Conjugation products of epinephrine with glycine, tyrosine, glutamic acid, urea, lactic acid, dextrose, cholesterol and cebione have been made by using liquid ammonia as a solvent and dispersing agent. All the products, except that with cebione, produce a more sustained rise in blood pressure than epinephrine; in this regard the products made with glycine, dextrose and cholesterol are most potent.

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References

1. Roberts, Coblens, Dyko and Cohen: Proc. Soc. Exp. Biol. and Med. 31, 998, 1934.
2. Fernelius and Johnson: J. Chem. Ed., 6, 444, 1929.
3. Aihara, Sakiji and *Ito*, Hideo: Osaka IgK.Z. 35, 1493 (1936); Japan. J. Med. Sci. *IV*. Pharmacol. 10, Abstracts, 47.
4. Huggins, M. L.: J. Organic Chemistry, 1, 407, 1936.