Reversal by sotalol of the respiratory depression induced in mice by ethanol

Smith & Hayashida (1970) reported that treatment with a β-adrenoceptive antagonist inhibited respiratory depression in mice subsequently given graded doses of ethanol, and correlated with this effect was a shortening of the sleeping time (Smith, Hayashida & Kim, 1970). While prevention of ethanol intoxication is of interest, reversal of the central depression would imply a therapeutic potential.

Swiss-Webster female mice, 18–22 g, had food and water available up to the time of the experiment. Groups of at least 10 mice for each dose were then injected with ethanol as a 25% solution (w/v) in normal saline, in doses ranging from 1 to 5 g/kg, The groups receiving only ethanol solution were tested 45 min after injection. An incision was then made in the ventral surface of the proximal third of the tail and the capillary blood collected into 125 μl heparinized tubes without significant air exposure. The contents of the tube were then transferred to a Radiometer microelectrode system for determination of the pH and pCO_2.

A dose-related fall in pH with a concomitant rise in pCO_2 was found (Fig. 1). The horizontal bars indicate the standard error. The curves are plotted in a linear fashion to allow comparison with normal values and the influence of the drug on control pH or pCO_2. Sotalol, (MJ 1999) 10 mg/kg, was given in aqueous solution 15 min after ethanol, at a time when many mice injected with ethanol were already asleep. Thirty min later capillary blood was obtained from the tails. Mice develop maximal respiratory depression about 30 min after parenteral injection of a moderate dose of ethanol. The pH and pCO_2 changes begin within minutes after ethanol injection. In the mice treated with sotalol, capillary blood pH did not fall except at the highest doses of ethanol, while pCO_2 rose only slightly.

The fact that mice treated with large doses of ethanol were already narcotized at the time of sotalol injection suggests that the drug acts not by preventing entry of ethanol into the brain but by antagonizing some action of ethanol or possibly acetaldehyde. Since the respiratory depressive effect of ethanol is closely related to dose, whereas acetaldehyde concentration is probably constant, it would seem that ethanol alone depresses respiration.

The sleeping time was slightly attenuated in mice treated with ethanol (4 g/kg) and subsequently with sotalol (40 ± 3·9 min versus 30 ± 3·6 min). There was however, no shortening of sleeping time for mice given the larger dose of 5 g/kg despite relief of the respiratory depression and acidosis. Evidently respiratory depression and narcosis are not equivalents.

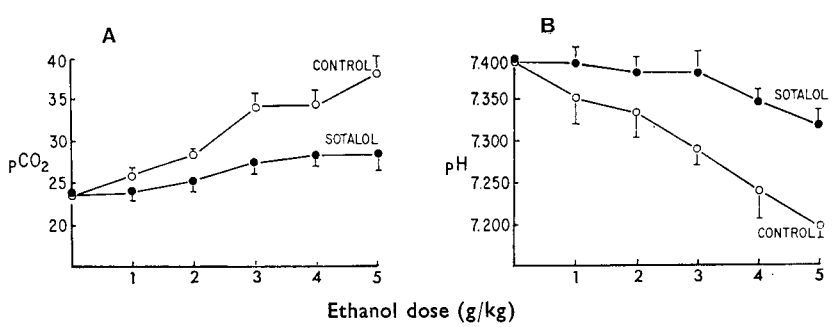

FIG. 1. Capillary blood pH and pCO_2 (mm) of mice 45 min after injection of graded doses of ethanol. Sotalol (MJ 1999) was given 15 min after ethanol. Horizontal bar represents s.e.

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The fluorimetric determination of thymoxamine in plasma

Thymoxamine hydrochloride, a specific competitive α-adrenoreceptor blocking drug, and its deacetylated derivative have fluorescent properties which may be exploited for the purposes of recognition and measurement in body fluids and in pharmaceutical formulations. Thymoxamine hydrochloride fluoresces in de-ionized aqueous solution, the pH for maximum fluorescence lying between 7–8. Fluorescence falls more steeply towards alkaline than acid pH and there is another peak at pH 1–1·5 (Fig. 1). Desacetylthymoxamine hydrochloride has the same excitation and emission wavelengths as thymoxamine hydrochloride.

The pK_a values of thymoxamine were found to be about 8·6 and 2·0. Concentrations between 100 ng to 50 μg extracted from water gave a straight line relationship with fluorescence, beyond which quenching occurred. No change in fluorescence of the extract was observed for up to 24 h at room temperature (20°), while the aqueous solution continues to increase in fluorescence for at least this length of time. Benzene with 1·5% isoamylalcohol gave the most consistent recovery of the drug from standard aqueous and plasma solutions, when compared with heptane, chloroform and ethyl acetate. Materials used were:

Thymoxamine hydrochloride 50 mg capsules, solution for intravenous injection 5 mg/ml and powder (Opilon), (William Warner & Co.); desacetylthymoxamine hydrochloride powder, (William Warner & Co.); benzene and isoamylalcohol, Grade A, Analar, (May & Baker Ltd.); sodium hydroxide solution, 1 N in deionized water; and hydrochloric acid, 0·1 N in de-ionized water.

Standard thymoxamine solution was prepared from 10 mg of powder in 10 ml of de-ionized water.

Plasma (2·5 to 4 ml) separated from heparinized blood was extracted by shaking with 1 ml of 1N sodium hydroxide and 12 ml of benzene containing 1·5% isoamylalcohol for 10 min on an automatic shaker. After centrifugation, 10 ml of the supernatant organic layer were added to 1·5 ml of 0·1N hydrochloric acid in another set of stoppered centrifuge tubes for extraction of the drug into the acid phase by shaking for 10 min. After centrifugation, 1·2 ml of the acid phase from the bottom layer was transferred to test tubes and immersed in a boiling water bath for 30 min. The tubes were then cooled to room temperature and the fluorescence of the acid phase measured with an Aminco-Bowman spectrophotofluorimeter at maximum excitation 295 nm and maximum emission 335 nm (uncorrected).

For each subject, duplicate plasma samples were taken at different times before and after administration of the drug. Internal standards were obtained by adding known amounts of thymoxamine to plasma samples together with control blanks which did not contain the drug and these were treated in the same way.

A straight line relationship was obtained with concentrations between 0·2 and 1 μg of drug in plasma and its fluorescence (Fig. 2).