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INHIBITION OF ETHANOL METABOLISM *IN VIVO* BY 4-IODO-PYRAZOLE

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Abstract—In experiments in rats, the *in vivo* effect of 4-iodo-pyrazole on ethanol metabolism was studied. An ethanol dose of 32·6 m-mole/kg was administered. Blood samples were withdrawn at regular intervals, and ethanol analyzed by the Widmark method and by gas chromatography. 4-iodo-pyrazole in doses of 0·05 to 1·25 m-mole/kg inhibited the metabolism of ethanol from 19 to 84 per cent, and the presence of ethanol in blood was prolonged from 4 hr in the controls to 28 hr in the experiment with 1·25 m-mole/kg 4-iodo-pyrazole. 4-iodo-pyrazole is a potent ADH blocker, the ED_{50} being 0·27 m-mole/kg of the same order of magnitude as for pyrazole *in vivo*.

AN INHIBITORY effect of pyrazole on liver alcohol dehydrogenase (LADH) has been demonstrated *in vitro* by Theorell and Yonetani.[1] Investigations *in vivo* have elucidated the inhibitory effect of pyrazole on the metabolism of ethanol in the rat (Goldberg and Rydberg[2]).

In a comprehensive investigation on the properties of liver alcohol dehydrogenase, Theorell *et al.*[3] also found 4-iodo-pyrazole to be active as an inhibitor of ethanol metabolism *in vitro*, the inhibitory effect on a molar base being ten times that of pyrazole.

In a survey by Lester *et al.*[4] on the effects of various analogues of pyrazole on the metabolism of ethanol, 4-substituted compounds were described as active inhibitors of ADH *in vivo*. However, the effect of 4-iodo-pyrazole on ethanol metabolism was not investigated.

The aim of the present study was to elucidate (a) the interaction between 4-iodo-pyrazole and ethanol metabolism *in vivo* in rats with special reference to changes in the rate of elimination of ethanol from the blood, and (b) to compare the activity *in vivo* of 4-iodo-pyrazole to that of pyrazole on the metabolism of ethanol *in vivo*.

MATERIAL AND METHODS

Material.

Thirty-two female rats of the Sprague–Dawley strain, weighing 200–235 g.

Chemicals.

Ethanol in a 12% solution (w/v), in distilled water.

4-iodo-pyrazole in weighed doses was solved into a volume of absolute ethanol to obtain the desired ethanol dose, as the solubility of the substance in water is limited. Then distilled water was added until an ethanol concentration of 12% (w/v) was reached.

Pyrazole in a 2% (w/v) solution, in distilled water.

Ethanol sampling and analysis. Duplicate samples of 0·1 ml were withdrawn from the tip of the tail, at 30 min and then at 60 min intervals, after the administration of ethanol, until the blood ethanol concentration reached zero, for a total of 5 to 28 hr.

Ethanol was analyzed by the Widmark method[5] and by gas chromatography.[6]

Evaluation. From the blood ethanol curves, the *time in minutes* until ethanol reached zero was calculated, defined as the x-intercept of the rectilinear regression curve.

In the experiments with 4-iodo-pyrazole or pyrazole, the *degree of inhibition* of ethanol metabolism was calculated according to the following formula:

$$\text{Degree of inhibition} = 100 \times \frac{\text{minutes}_{\text{4-iodo-pyrazole}} - \text{minutes}_{\text{control}}}{\text{minutes}_{\text{4-iodo-pyrazole}}} \quad (1)$$

RESULTS

1. *Control experiments.* A typical experiment is shown in Fig. 1a, when 32·6 m-mole/kg ethanol was given i.p. Blood ethanol concentration was followed for 5 hr. The rectilinear decline of the curve is evident. The mean time until blood ethanol reached zero was 255 min.

2. *4-iodo-pyrazole and ethanol.* The effect of 4-iodo-pyrazole is illustrated in Fig. 1b,

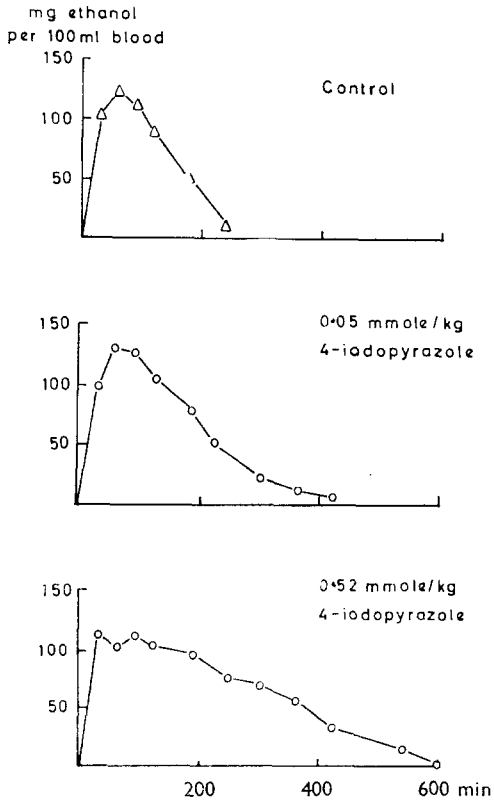

FIG. 1. Blood ethanol values after 32·6 m-mole/kg ethanol in 12% (w/v) solution administered i.p. in rat. (a) control; (b) 0·05 m-mole/kg 4-iodo-pyrazole; (c) 0·52 m-mole/kg 4-iodo-pyrazole.

0·05 m-mole/kg 4-iodo-pyrazole being administered with 32·6 m-mole/kg ethanol. The presence of ethanol in the blood is prolonged, in this case to ca. 420 min.

In Fig. 1c the effect of a higher dose of 4-iodo-pyrazole, 0·52 m-mole/kg, is shown. The duration of ethanol in the blood was in this experiment 610 min.

3. *Pyrazole and ethanol.* The experiments performed in the same strain of rats with pyrazole and the same dose of ethanol were used for comparison.

4. *Degree of inhibition.* The degree of inhibition of ethanol metabolism brought about by various doses of 4-iodo-pyrazole was calculated according to formula (1) and set off against the dose of 4-iodo-pyrazole administered (Fig. 2). In the doses

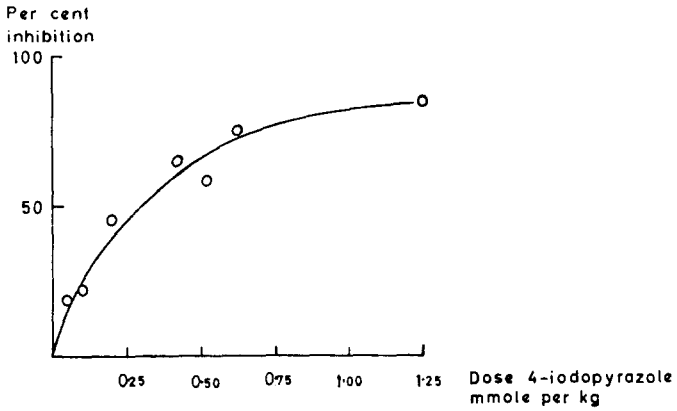

FIG. 2. Inhibition of ethanol metabolism *in vivo* after administration of various doses of 4-iodo-pyrazole in rat, ethanol dose 32·6 m-mole/kg.

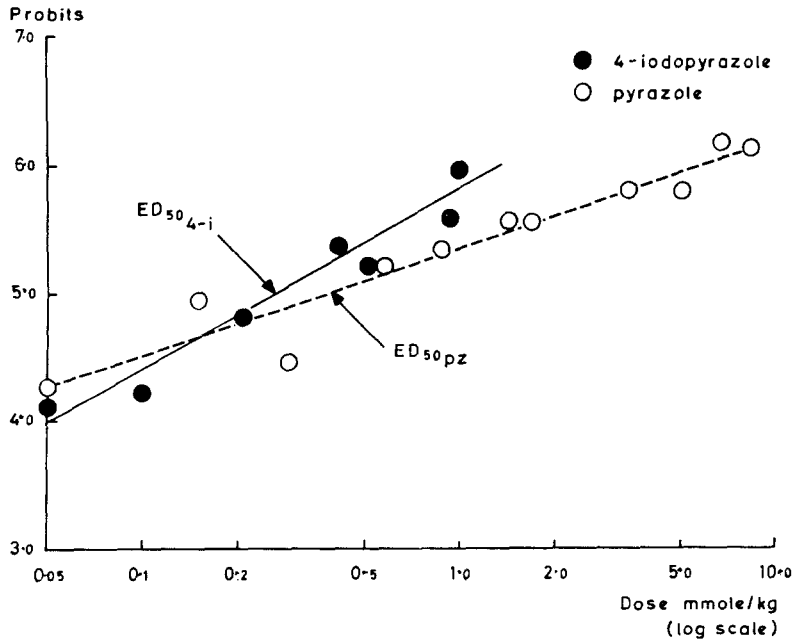

FIG. 3. Inhibition of ethanol metabolism *in vivo* by 4-iodo-pyrazole or pyrazole, ethanol dose 32·6 m-mole/kg. Per cent inhibition transformed to probits, and dose to log dose.

given, 0·05 to 1·25 m-mole/kg, the degree of inhibition calculated varied between 19 and 84 per cent.

Transformation of the degree of inhibition to probits and dose to log dose, according to Bliss[7] and Burn *et al.*[8] yielded rectilinear dose–response curves, allowing of calculating the ED_{50}. The dose of 4-iodo-pyrazole which inhibited the elimination of ethanol to 50 per cent was 0·27 m-mole/kg. The ED_{50} for pyrazole was of the same order of magnitude or 0·43 m-mole/kg.

One possible explanation for the discrepancy in activity of 4-iodo-pyrazole *in vitro* and *in vivo* may be due to different pH of the solutions of pyrazole and 4-iodo-pyrazole administered, to differences in absorption or to differences in distribution within the organism. This factor has not been further elucidated.

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