*Br. J. Pharmac.* (1971), **43**, 67–73.

# Interaction effects of ethanol and pyrazole in laboratory rodents

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**Summary**

1. Interactions of pyrazole and ethanol were studied in three laboratory test procedures. They included sleeping time in mice, rotor rod balance in rats and lever pressing behaviour of rats.

2. Equimolar concentrations of pyrazole and 3-methylpyrazole were compared for effects on enhancement of ethanol's activity on rotor rod holding time of rats.

3. Minimally effective doses of pyrazole, the LADH inhibitor, and 3-methylpyrazole, a non-inhibitor of LADH, when administered before ethanol, resulted in an increased behavioural depression. These interaction effects are probably not caused by inhibition of LADH but rather by an increase in the direct depressant action of either one or both of the compounds.

## Introduction

Pyrazole inhibits the metabolism of ethanol both *in vitro* and *in vivo*. In 1963, Theorell & Yonetani reported that pyrazole inhibited liver alcohol dehydrogenase (LADH) by formation of a ternary complex with LADH and nicotinamide-adenine dinucleotide (NAD). Lester, Keokosky & Felzenberg (1968) and Goldberg & Rydberg (1969) showed that pyrazole inhibited ethanol metabolism in rats as evidenced by the persistence of high blood alcohol concentrations. In the former study, it was reported that pyrazole and 4-substituted pyrazoles inhibited ethanol metabolism, while substitution in any other position did not produce a similar inhibition. More recently, two investigations (Bustos, Kalant, Khanna & Loth, 1970; Morgan & DiLuzio, 1970) appeared which dealt with the induction of fatty livers in animals treated with pyrazole-ethanol. Although the principal findings of these studies were distinctly controversial, the investigators were in agreement with regard to one important observation: the concentrations of blood ethanol in rats treated with pyrazole-ethanol persisted at a high level for as long as 16 or 20 h, thus indicating a pyrazole-induced inhibition of ethanol metabolism. Morgan & DiLuzio (1970) reported that ethanol intoxication appeared to be 'particularly pronounced' in the pyrazole-ethanol group of rats.

Although the above cited studies established that pyrazole decreases the rate of ethanol metabolism, resulting in a prolonged elevation of blood ethanol, the measures of concomitant intoxication were limited to gross behavioural observation.

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Our study investigated pyrazole-ethanol interactions in three psychopharmacologic testing procedures.

Upon completion of the study, a second experiment was conducted in which equimolar concentrations of pyrazole and 3-methylpyrazole were compared for effects in ethanol treated rats. The purpose of the experiment was to determine whether the results obtained in experiment 1 were related to an inhibition of ethanol metabolism or rather to a possible additive effect of pyrazole and ethanol.

## Methods

### *Experiment 1*

The tests used to evaluate pyrazole-ethanol interactions were sleeping time, rotor rod and an operant testing procedure.

The sleeping time test has been described in detail (Kakihana, Brown, McClearn & Tabershaw, 1966). The measure of sleep time refers to the time period between the loss and regaining of the righting reflex by a mouse. An arbitrary cut-off time was set for sleeping time; if an animal did not right itself within 2·5 h after injection, a score of 150 min was assigned to its sleeping time.

Male, white mice averaging 20 g in weight were assigned to groups consisting of ten animals each. Each group was given an intraperitoneal injection of saline or pyrazole 30 min before an intraperitoneal injection of ethanol. Pyrazole-ethanol doses were 0·24+2·5, 0·29+3·3, 0·34+4·0 or 0·44+5·5 g/kg, respectively. The pyrazole dose was selected so that g/kg ethanol equalled mmol/kg pyrazole plus 1 mmol, thereby assuring as complete an inhibition of ethanol metabolism as was possible (D. Lester, personal communication).

The rotor rod apparatus (Dunham & Miya, 1957) consisted of an electric motor which rotated at four revolutions per minute. The motor drove a metal rod (24 inches long x $\frac{1}{4}$ inch diameter) mounted 13 inches above an electrified grid floor to discourage animals from jumping off the rod. Seven male, Sprague-Dawley rats of Holtzman strain were trained to maintain balance on the rotating rod until able to remain on for a minimum of 55 s on each of ten consecutive trials. On non-drug days the rats were given five test trials in order to maintain stability of the holding response. On drug days the animals were deprived of food and water for 18 h and were given ten trials before and after drug administration. Rats received either saline, saline and ethanol, pyrazole or pyrazole plus ethanol. Pyrazole (0·24 g/kg) and saline were given intraperitoneally, while ethanol (2·5 g/kg) was administered orally. This ineffective dose of ethanol was selected since data from the sleep time test showed a pyrazole-induced enhancement of ethanol's effects. Drug administrations were spaced at least 2 weeks apart. The experimental sessions were conducted 2·5 h after saline or pyrazole, and 2 h after ethanol administration.

In the operant test procedure, three rats were gradually reduced to 80% of original body weight. This was accomplished by limiting food intake to one Wayne Lab Blox pellet per day until the desired weights were reached. They were maintained at these weights by limited feedings after each experimental session. Then the rats were trained to press a lever for a liquid food reward obtainable on a 2 min variable interval schedule of reinforcement (Ferster & Skinner, 1957). Experimental sessions of 1 h duration were conducted at the same time each day from Monday to Friday

inclusive each week. When lever pressing rates for all animals reached at least twenty responses/minute for 5 consecutive days, the experiment was begun.

During week one, the rats were given saline followed 30 min later by ethanol at 0·75, 2·5 or 4·5 g/kg, 15 min before the experimental session. All injections were by the intraperitoneal route. The doses of ethanol used were minimally effective doses as determined in preliminary experiments. During week three, two of the rats were given the same ethanol dose 30 min after a dose of pyrazole. For a third rat (39), this treatment was given during the fourth week. Three weeks after receiving pyrazole and ethanol, each rat was given saline and ethanol. During the ninth week, two of the rats received distilled water and pyrazole in the dose previously administered. For rat 39 this treatment was given during the tenth week.

*Experiment 2*

The rotor rod test was used as described in experiment 1. During the first week, five Sprague-Dawley rats were treated with 0·24 g/kg (3·5 mmol/kg) pyrazole and five rats were treated with 0·29 g/kg (3·5 mmol/kg) 3-methylpyrazole administered intraperitoneally. These doses of pyrazole and 3-methylpyrazole, when administered previously in combination with distilled water, were without effect on rotor rod performance. Thirty minutes later, the rats were given an oral dose (2·5 g/kg (54 mmol/kg)) of ethanol (30% v/v solution). The rats were tested on the rotor bar at 2, 12 and 20 h after ethanol administration. Blood samples obtained from tail veins at the same time intervals were analysed for ethanol by a gas chromatographic procedure previously described (Wallace & Dahl, 1966). During the second week, the rats were given saline intraperitoneally followed 30 min later by a 2·5 g/kg oral dose of ethanol. Rotor rod testing and blood sampling and analysis were performed as during the first week. During the third week, rats that had received pyrazole previously were given the 3-methylpyrazole-ethanol combination and rats that had received the 3-methylpyrazole previously were given the pyrazole-ethanol combination. Rotor rod testing and blood sampling and analysis were as during the first week. All animals were deprived of food and water 18 h before and during the testing periods.

**Results**

Figure 1 shows that all except the lowest dose of pyrazole augmented ethanol's effects on sleep time significantly above control values ($P<0·001$). After pyrazole and ethanol, mice slept much longer than control animals treated with saline plus ethanol.

Figure 2 includes mean rotor rod holding time data for seven rats. Saline or pyrazole were without effect, while saline plus ethanol produced a slight but insignificant reduction of rotor rod holding time. However, when pyrazole was administered before ethanol, a rather precipitous drop in holding time occurred. Rats were unable to maintain rotor rod balance when an ineffective dose of ethanol was preceded by pyrazole. These data were significant at the $P<·001$ level by Student's $t$ test.

Figure 3 shows the effects of each drug condition on the variable-interval response rates. Response rates under each condition are expressed as a percentage of the pre-drug control rate. Saline and ethanol reduced response rates by 20–25% in rat 57,

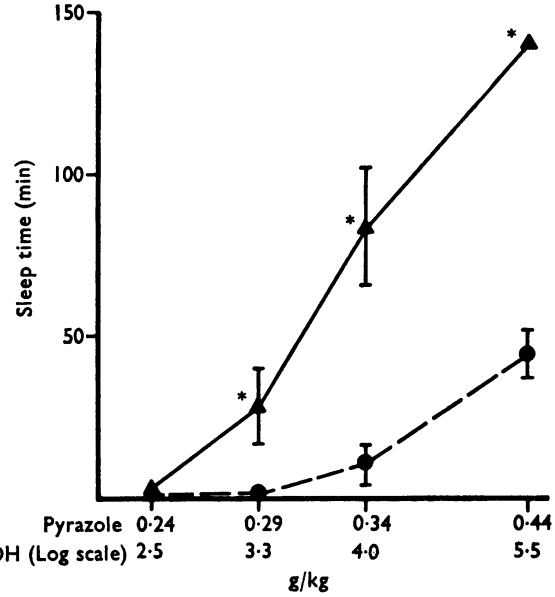


FIG. 1. Effect of ethanol in combination with saline and pyrazole on sleep time induction in mice. Standard errors are indicated by vertical bars. Since six of the eight animals slept past the cut-off time of 150 min when administered pyrazole before the 5·5 g/kg ethanol, no standard error was computed for that point. The number of animals used varied from eight to ten on each point. Pyrazole+ETOH (▲——▲); saline+ETOH (●- - -●). $P<0·001$ compared with control.

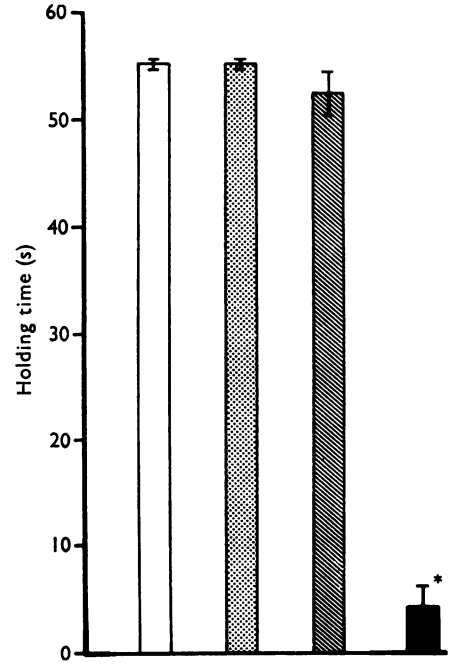


FIG. 2. Effect of ethanol in combination with saline and pyrazole on the rotor rod performance of rats. Average data are for seven animals. Standard errors are indicated by the vertical bars . Saline (□); pyrazole, 0·24 g/kg (⁘); saline+ETOH, 2·5 g/kg (▨); pyrazole, 0·24 g/kg+ETOH, 2·5 g/kg (■). * $P<0·001$ ($n=7$) compared with control.

but were virtually without effect on response rates of rats 39 and 33. Pyrazole and distilled water also had no effect on response rates of rats 39 and 33 and only a slight depressant effect on the rate of rat 57. However, when pyrazole was given before ethanol, there was a significant reduction of response rates for all of the rats.

The results of experiment 2 are shown in Table 1. When animals were treated with saline before ethanol at 2·5 g/kg rotor rod holding time was virtually unaffected at 2, 12 or 20 h after ethanol. Blood alcohol concentrations (BAC) were 101 ± 8·7 mg% at 2 h and 0% at 12 and 20 h after ethanol, indicating complete metabolism of ethanol. The 3-methylpyrazole-ethanol combination produced some reduction

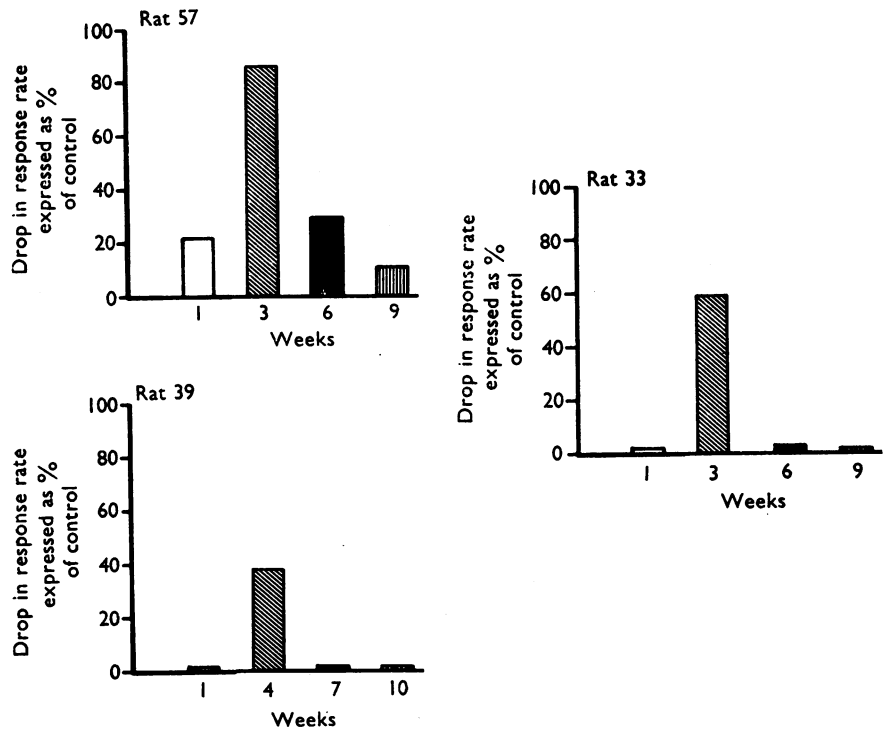


FIG. 3. Effect of ethanol alone and in combination with saline and pyrazole on variable interval lever response rates of rats. Rat 57: ETOH, 4·5 g/kg+saline (□) and (■); ETOH, 4·5 g/kg+pyrazole 0·37 g/kg (▩) and (■); distilled water+pyrazole, 0·37 g/kg (|||). Rat 33: ETOH, 0·75 g/kg+ saline (□) and (■); ETOH, 0·75 g/kg+pyrazole, 0·10 g/kg (▩); distilled water+pyrazole, 0·10 g/kg (|||). Rat 39: ETOH, 2·5 g/kg+saline (□) and (■); ETOH, 2·5 g/kg+pyrazole, 0·24 g/kg (▩); distilled water+pyrazole, 0·24 g/kg (■).

TABLE 1. *Rotor bar holding time and concentrations of blood ethanol after pretreatment with 3-methylpyrazole and pyrazole*

| | Rotor bar holding time (s)* | | | Blood alcohol concentrations (mg%)† | | |
|---|---|---|---|---|---|---|
| Drug treatment | 2 h | 12 h | 20 h | 2 h | 12 h | 20 h |
| Saline+ethanol (54 mmol/kg) | 54±0·7 | 55±0·0 | 55±0·0 | 101±8·7 | 0±0·0 | 0±0·0 |
| 3-Methylpyrazole (3·5 mmol/kg)+ Ethanol (54 mmol/kg) | 34±5·9 | 54±0·2 | 55±0·0 | 34±9·1 | 3·2±1·6 | 0±0·0 |
| Pyrazole (3·5 mmol/kg)+ Ethanol (54 mmol/kg) | 15±7·0 | 6±4·0 | 55±0·0 | 82±12·2 | 77±8·2 | 41±4·7 |

*Values represent mean data for ten rats. †Values represent mean data for eight rats.

in rotor rod holding time only at 2 h after ethanol ($P<0.01$). BAC for this group was depressed considerably below that of the saline-ethanol rats ($P<0.001$). Rats treated with pyrazole-ethanol attained a BAC of $82 \pm 12.2$ mg% at 2 h and a value of $77 \pm 8.2$ mg% at 12 h after ethanol. At 20 h after ethanol the BAC was still relatively high at $41 \pm 4.7$ mg% ($P<0.001$). Rotor rod holding time was reduced considerably at 2 and 12 h ($P<0.001$) and fully recovered at 20 h after ethanol.

**Discussion**

These data illustrate that treatment of laboratory animals with pyrazole, the LADH inhibitor, before a non-effective dose of ethanol, results in a depressant action which is characteristic of ethanol alone at higher doses. This finding might reflect a pyrazole-induced enhancement of ethanol's depressant action and as such is in accord with the recent work of Lelbach (1969) who found that inhibition of alcohol dehydrogenase (ADH) by threshold doses of pyrazole during chronic ethanol administration augmented ethanol induced mortality and produced hepatotoxicity in rats. A common mechanism of action might underlie the findings of both studies since for most pharmacologically active molecules, potency increases are usually accompanied by concomitant increases in toxicity.

The doses of pyrazole used in this study were selected so as to inhibit the *in vivo* metabolism of ethanol by at least 90% (D. Lester, personal communication). Therefore, the depressant activity in these three experimental procedures, if due to an enhancement of ethanol's activity, would probably be attributable to a direct action of ethanol rather than to its principal metabolite, acetaldehyde. A similar suggestion regarding the addictive characteristics of ethanol was made by Mendelsohn (1970) who stated, 'it appears that the major addictive agent in alcoholism is ethanol itself and not its major metabolite.' Although it seems reasonable to interpret the findings of this experiment in terms of a pyrazole induced enhancement of ethanol's depressant activity, the design of our experiment does not allow one to rule out an alternative possibility. Perhaps the results might be due to an ethanol enhancement of the direct depressant action of pyrazole.

Results of experiment 2 indicate that impairment of rotor rod holding time is not directly related to concentrations of blood ethanol. Rotor rod holding time was impaired 2 h after ethanol in the pyrazole-ethanol and 3-methylpyrazole-ethanol rats, but not in the saline-ethanol animals. Blood alcohol concentrations were high for the saline-ethanol and pyrazole-ethanol rats, but not for the 3-methylpyrazole animals. Twelve hours after ethanol the rotor rod holding time was impaired only in the pyrazole-ethanol animals and high BAC's persisted in this group. These data may indicate a potentiation of ethanol's depressant action by the pyrazole moiety, a potentiation of the depressant action of pyrazole type compounds by ethanol, or a summation of the depressant action of ethanol and pyrazole type compounds. Preliminary data of a current experiment indicate that similar interaction effects may be obtained with barbiturates. Treatment of animals with pyrazole before a subeffective dose of sodium barbitone also produces an impairment of rotor rod holding time.

It is of interest to note that at 2 h, BAC's after 3-methylpyrazole and ethanol were depressed considerably below the BAC's for the saline-ethanol rats. This unexpected finding suggests the possibility of an inhibition of ethanol absorption by 3-methylpyrazole and provides the basis for a study currently in progress.

We thank Dr. David Lester for his most helpful comments concerning the pyrazole dose, and wish to acknowledge the capable assistance of James Polonis and Richard Marin. Our thanks are due to Jack Bell for carrying out the blood-alchohol determinations. This research was supported by USPHS Research Grant MH 15922.

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(*Received January* 6, 1971)