Interactions of Pyrazole and Ethanol on Norepinephrine Metabolism in Rat Brain[1]

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ABSTRACT

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Single large doses of pyrazole (100–200 mg/kg) given either i.p. or orally caused a decrease in brain norepinephrine and 3-methoxy-4-hydroxyphenylethylene glycol sulfate, but no change in dopamine β-hydroxylase activity. The effects were enhanced by daily administration for 3 to 4 days. Concomitant administration of ethanol prevented the effects. With smaller doses (50 mg/kg/day) given orally for several days, there was little or no change for 3 days, but after 6 days there was an increase in both parameters. Dopamine β-hydroxylase activity

was also increased. The daily administration of ethanol alone (6.0 g/kg/day) for 6 days caused increased norepinephrine, but 3-methoxy-4-hydroxyphenylethylene glycol sulfate was diminished and dopamine β-hydroxylase was unaffected. When the two drugs were given simultaneously, steady-state levels of norepinephrine were unaltered, but the sulfate metabolite was increased as was dopamine β-hydroxylase. The results suggest that pyrazole and/or ethanol, administered daily for 6 days, leads to adaptive responses in catecholamine metabolism. Pyrazole (or a metabolite) has marked effects of its own, some or all of which are independent of its effects on alcohol dehydrogenase. When the two drugs are administered together, it is difficult to know whether the observed changes are independent or overlapping effects.

Pyrazole inhibits alcohol dehydrogenase and has been used extensively for this purpose in studies of ethanol metabolism. One prime example of such work is that of Goldstein (1975) who used the drug as a part of a model for alcohol dependence and withdrawal. Others have used pyrazole in efforts to distinguish between alcohol dehydrogenase and alternate routes of ethanol oxidation (Lieber et al., 1975; Lieber and Dicarli, 1972). Both of these approaches are somewhat controversial (Thurman et al., 1975), particularly in view of the known

synergistic effect of pyrazole on alcohol intoxication (Blum et al., 1971; Goldberg et al., 1972; LeBlanc and Kalant, 1973). The enhancement of alcohol intoxication by pyrazole has been attributed to changes in brain monoamine concentrations (Littleton et al., 1974), which along with their major metabolites are reported to be increased after chronic administration of ethanol (Post and Sun, 1973; Karoum et al., 1976; see, however, Pohorecky, 1974; Hunt and Majchrowicz, 1974). On the other hand, MacDonald et al. (1975) found that pyrazole per se caused a dramatic decrease in brain norepinephrine (NE) with little or no changes in dopamine or serotonin. They suggest that pyrazole inhibits dopamine β-hydroxylase (DBH) in vivo. This explanation is inconsistent with our own observa-

tion that pyrazole and other nitrogen-containing heterocyclics stimulate DBH *in vitro* (Brown and Harralson, 1976). Efforts to delineate these ambiguities and to gain a deeper insight into the interaction of these two drugs led to the observations reported in this article.

Methods

White male rats (Sprague-Dawley), weighing from 165 to 198 g at the start of the experiment, were used. They were housed before and during the experiment in cages of five each under artificial light with alternating periods of darkness (12:12 hours, respectively). Animals were selected at random, paired as to weight, and divided into groups. Injections were performed at 9:00 to 11:00 A.M. Animals were weighed daily from the beginning of the experiments, and just before they were sacrificed by decapitation. Blood samples were taken during exsanguination.

Pyrazole. In one series of experiments, pyrazole was injected i.p. (200 mg/kg/day) into three groups of rats for 1, 2 and 4 days, respectively. The drug was administered initially and every 24 hours as a 2.5% solution in 0.9% saline. Controls were done simultaneously and received only the saline. Each group was sacrificed 24 hours after the final dose.

Pyrazole and ethanol. Ethanol and pyrazole were administered separately or simultaneously by intubation. In these experiments, some of which involved using large doses of ethanol with or without pyrazole, the oral route was chosen for purposes of convenience. The dosage of pyrazole was reduced in order to minimize deleterious effects on weight and other physiological parameters (MacDonald *et al.,* 1975; see also"Results"). Unless specified otherwise, pyrazole was given (50 mg/kg/day) as a 0.2% solution in saline, and ethanol (6.0 g/kg/day) was administered as a 30% solution in saline. Controls were done simultaneously and received equal volumes of saline. Sacrifice was performed 24 hours after the final dose.

In some experiments, ethanol was administered in a single dose (6.0 g/kg) and the animals were sacrificed at various hourly time intervals.

Enzyme analysis. For DBH analyses, the brain and adrenals were removed, weighed and homogenized in Tris-HCl buffer (pH 6.0) containing 0.2% Triton X-100 (Coyle and Axelrod, 1972), and were then assayed essentially as specified by Henry *et al.* (1975). Copper was used to negate the effects of endogenous inhibitors. Before the beginning of the experiment, it was necessary to determine the optimum level of copper for each tissue (Molinoff *et al.,* 1971) and also to determine that pyrazole did not interfere with the interaction of copper and tissue inhibitors. Serum DBH activity was determined as specified by Henry *et al.* (1975).

Catecholamine determination. Tissues to be analyzed for catecholamines were removed quickly and frozen immediately in a dry-ice-ethanol mixture. NE was determined by the procedures of Anton and Sayre

(1962). A fluorometric procedure described by Meek and Neff (1972) was used to analyze 3-methoxy-4-hydroxyphenylethylene glycol sulfate (MHPG-SO₄). Recoveries of internal standards were 90 ± 6 and 82 ± 8%, respectively. Recent data have confirmed that glycol sulfates are the major metabolites of NE in brain (Brown and Zawad, 1977; Karoum *et al.,* 1976), and their use as an index to changes in NE turnover has been proposed (Stone, 1976; Karoum *et al.,* 1976; Meek and Foldes, 1973). Gas liquid chromatography was used to determine plasma alcohol concentrations (PAC) (Sargent *et al.,* 1974). Data were analyzed statistically using Student's *t* analyses. All values of P less than .05 were considered significant. Other conditions are given in the legends to the tables.

Results

Effects of pyrazole. The extensive effects of pyrazole and its derivatives on physiological parameters such as body weight and temperature are well known (MacDonald *et al.,* 1975; Maynussen *et al.,* 1972). We have observed some of these changes reported previously. Animals receiving pyrazole appeared narcotized. They were somnolent and flaccid, but responded to moderate stimulus. The intensity and duration of these symptoms increased with dosage in the range of 50 to 500 mg/kg i.p. After 2 days, animals receiving pyrazole (200 mg/kg i.p.) had lost an average of 8.6% of their initial weight; controls had gained 7.0%. After 4 days, the weight loss was increased to 19% and the control gain was about 10%. In confirmation of the observations of MacDonald *et al.* (1975), we found no change in brain weight but a marked increase in the weight of the adrenal glands. The gain in the latter was apparent after 2 days (25%); after 4 days, the gain had increased to about 34%. No such changes were observed, however, with a large single dose of pyrazole over a period of less than 24 hours, or with smaller doses (50 mg/kg) given by intubation over a period of several days. On a per gram basis, no change occurred in adrenal (4130 *vs.* 4190 units) or brain (90.6 *vs.* 90.7 units) DBH activity.

After 4 days of pyrazole (200 mg/kg/day i.p.), DBH activities were still unaffected in the brain and adrenals. In serum, enzyme activity declined from 19.2 ± 1.4 to 12.2 ± 0.5 units, with *N* values of 14 and 18, respectively. The change in serum DBH levels could not be detected after 24 hours, but had occurred after 48 hours. Studies on these and related observations on alterations in serum DBH by pyrazole will be presented in a separate report (Harralson *et al.,* 1978).

The data in table 1 confirm the observations of MacDonald *et al.* (1975) that brain NE con-

centrations are depleted after pyrazole. In addition, the concentrations of MHPG-SO$_4$ were also decreased.

Effects of ethanol. Measurements made 24 hours after a single large oral dose of ethanol showed that brain NE was increased. The MHPG-SO$_4$ concentrations were unchanged as compared to controls. These results were surprising since PAC had returned to normal after

TABLE 1

Effects of pyrazole on brain concentrations of NE and MHPG-SO$_4$*

Dose	Time	NE	MHPG-SO$_4$
mg/kg	*hr*	*μg/g*	
0	24–96	0.234 ± 0.011 (5)	0.168 ± 0.009 (6)
200	24	0.170 ± 0.008 (5)	
200	48		0.116 ± 0.009 (6)
200	96	0.099 ± 0.057 (4)	0.044 ± 0.002 (6)

* Values for NE and MHPG-SO$_4$ have been corrected for percent recovery of internal standards. They are expressed as means ± S.E.; (N) = number of animals. Dashes are used to indicate that no analyses were done. Pyrazole was injected i.p. daily as a 2.5% solution in 0.9% saline, and the animal was sacrificed 24 hours after the final dose. Controls were injected with an equal volume of saline. The experimental data above are significantly different from controls, $P \leq .001$. Other details are given in the text.

about 10 hours. The experiments with ethanol were repeated and extended therefore to cover time intervals ranging from 0 to 24 hours. Steady-state levels of NE and MHPG-SO$_4$ were determined at selected intervals during this time. The results are shown in figure 1.

In the controls there was a rapid increase in NE and MHPG-SO$_4$ within the 1st hour after intubation. No further changes were observed up to 24 hours. In these rats, but not in those receiving ethanol, a zero time point was determined by sacrificing a group about 10 seconds or less after intubation.

In ethanol-treated animals, the steady-state levels of NE remained relatively constant for at least 6 hours, but were significantly increased at 12 hours. Within the 1st hour, MHPG-SO$_4$ tended to increase, but the change was not statistically significant. After 3 hours, MHPG-SO$_4$ was decreased and continued to decline until it reached a minimum value of about 50% of control concentrations at about 12 hours. Unlike the alterations in NE, MHPG-SO$_4$ had returned to control levels by 18 hours. Interestingly, the time-dependent increase in NE, and the MHPG-
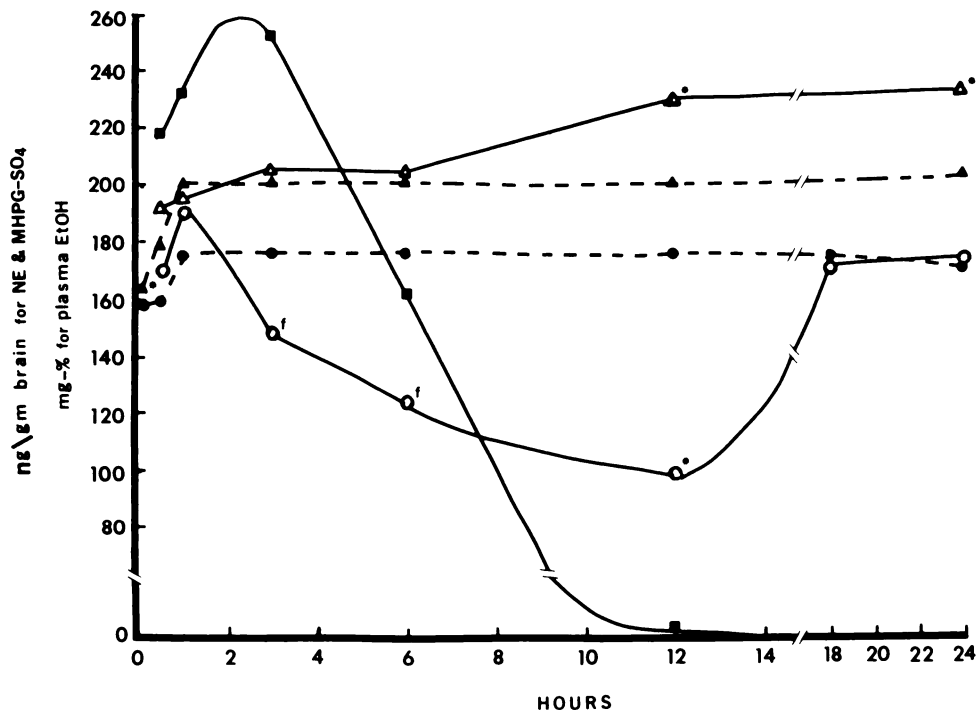

Fig. 1. Effects of ethanol on NE metabolism in rat brain. Ethanol (6 g/kg) or saline was administered by intubation and the rats were sacrificed at various time intervals. Each point represents the mean value of analyses from 5 to 6 rats (total $N = 61$). Data from animals that received ethanol are represented by ——; controls by – – –. Analytical results are represented as follows: NE, △ (controls, ▲); MHPG-SO$_4$, ○ (controls, ●); PAC, ■. Other experimental details are presented in the text. Significant difference from controls is indicated by * ($P < .01$) and ^{f} ($P < .05$).

TABLE 2

Effects of oral doses of pyrazole and ethanol on norepinephrine metabolism in rat brain

Pyrazole (50 mg/kg) and ethanol (6.0 g/kg) were administered once daily (9:00 A.M.) for 3 days (A) or 6 days (B), either separately or together by intubation. Animals were sacrificed 24 hours after the final dose. Blood alcohol concentrations in those animals receiving both drugs simulatenously were 47 ± 15 mg/100 ml; in those animals receiving ethanol alone, there was only a trace.

One DBH unit equals 1 micromole of octopamine produced per gram per hour.

Drug	NE	MHPG-SO$_4$	DBH
	$\mu g/g$		units
A. Saline (6)	0.228 ± 0.003	0.156 ± 0.004	87.7 ± 1.8
Pyrazole (4)	0.225 ± 0.007	0.136 ± 0.009 [a]	93.6 ± 1.9
Ethanol (7)	0.278 ± 0.008 [b]	0.124 ± 0.014 [a]	80.1 ± 2.9
Ethanol plus pyrazole (8)	0.233 ± 0.003	0.156 ± 0.008	85.9 ± 2.6
B. Saline (6)	0.234 ± 0.005	0.159 ± 0.005	88.4 ± 1.4
Pyrazole (6)	0.292 ± 0.019 [c]	0.168 ± 0.015	106.0 ± 3.0 [c]
Ethanol (6)	0.318 ± 0.023 [b]	0.143 ± 0.005 [a]	87.9 ± 4.7
Ethanol plus pyrazole (6)	0.235 ± 0.20	0.188 ± 0.010 [a]	104.0 ± 2.4 [c]

[a] Significantly different from saline controls, $P < .05$.
[b] Significantly different from saline controls, $P < .001$.
[c] Significantly different from saline controls, $P < .01$.

SO$_4$ recovery, did not occur until the PAC was reduced to low values or zero.

In summary, the data in table 1 show that the administration of pyrazole caused decreases in brain NE and MHPG-SO$_4$. Ethanol also caused an alteration in these parameters and the changes were shown to be time-dependent (fig. 1). When the two compounds were administered simultaneously in a single dose by intubation (100 mg/kg and 6.0 g/kg, respectively), the concentrations of NE and MHPG-SO$_4$ were unaltered from the controls (data not presented).

Effects of pyrazole plus ethanol. The data in table 2 show the results of administering multiple daily doses of ethanol and pyrazole, separately or simultaneously. After 3 days, ethanol caused an increase in brain NE concentrations and a marked reduction of MHPG-SO$_4$. With lower doses (50 mg/kg) of pyrazole administered alone each day for 3 days, there was no apparent effect on NE, but MHPG-SO$_4$ concentrations were diminished. The two drugs appeared to counteract each other when they were given concomitantly. When the experiment was continued over a period of 6 days, ethanol again caused an increase in NE and a concomitant decrease in MHPG-SO$_4$. However, pyrazole alone now also caused an increase in NE, and for the first time in these experiments, MHPG-SO$_4$ was unchanged or increased. Furthermore, and also for the first time, the DBH activity of the brain was significantly increased. Under these conditions, the two drugs appeared to counteract each other with respect to NE, but MHPG-SO$_4$ was still significantly increased.

Discussion

Effects of pyrazole. Although it did not alter steady-state levels of dopamine (MacDonald *et al.,* 1975), an acute dose of pyrazole caused a decrease in NE and MHPG-SO$_4$ in the brain. The drug did not inhibit DBH *in vitro* (Brown and Harralson, 1976), nor did it affect brain DBH activity, which incidentally was measured under optimal conditions that may not reflect the physiological state. After six daily doses, the effects were reversed; there was an increase in NE and MHPG-SO$_4$, accompanied by a 20% increase in DBH activity. The latency of the change in DBH, coupled with the nature of the assay which measures both the soluble and bound forms of the enzyme, suggests that induced synthesis had occurred. If this proves to be true, the original decrease in NE and MHPG-SO$_4$ is probably due to some sort of feedback inhibition of the enzyme. This hypothesis is based on the fact that pyrazole *per se* does not block NE catabolism. However, the slow decrease in brain NE (24 hours) plus the recovery, and what may be a time-dependent induction of DBH, are reminiscent of reserpine action (Reid and Kopin, 1975; Reis *et al.,* 1975).

Recent studies by Deis *et al.* (1977) in rats showed that pyrazole-3,4[^{14}C] was converted to a 4-hydroxy derivative which was excreted as a conjugate. The interaction of pyrazole and its main metabolite with DBH are the subject of a companion study done by us (Harralson *et al.,* 1978). The results indicate that the central effects of pyrazole may not be due to inhibition of

DBH by 4-hydroxypyrazole, but to some as yet unknown metabolite or metabolites. In any event, it is interesting to note that the pyrazole-induced concomitant decrease in NE and MHPG-SO$_4$ is consistent with the fact that NADPH-linked aldehyde reductases are not inhibited by pyrazole and that they mediate MHPG formation from NE in brain (Tabakoff and Erwin, 1970; Erwin et al., 1972; Deitrich and Erwin, 1975). An NADPH (NADH)-linked alcohol dehydrogenase from brain has been described (Raskin and Sokoloff, 1972), but it is markedly inhibited by pyrazole.

Effects of ethanol and pyrazole. The data in figure 1 show that a single large dose of ethanol caused an increase in NE that was preceded at least 3 hours by a decrease in MHPG-SO$_4$. Except for the lag in NE change, the observations are consistent with a decline in NE catabolism resulting from inhibition of aldehyde reductase, mediated perhaps by feedback from acetaldehyde-induced inhibition of aldehyde dehydrogenase (Ridge, 1963; Duncan and Sourkes, 1973; Turner and Hicks, 1975). When the PAC is reduced to zero, the inhibition is relieved and the MHPG-SO$_4$ returns to control level. This interpretation is supported by the data in table 2 which show an enhancement of the effects with multiple injections of ethanol. Also, in every case, the changes were reversed by pyrazole. The latter, of course, inhibits the oxidation of ethanol to acetaldehyde.

There are several interesting aspects of these data, however, which are not easily explained by this interpretation. One interesting point is that the NE increase coincides with the return of the PAC to control values. Karoum et al. (1976) have observed that PAC may be related to changes in catecholamine metabolism in brain, particularly in ethanol-dependent rats. Another interesting fact is the lag period before the increase in NE, and how the latter remains elevated while MHPG-SO$_4$ returns to control values. However, with multiple doses of ethanol, the MHPG-SO$_4$ does not return to control levels (table 2). If the decrease in MHPG-SO$_4$ resulted from inhibition of the aldehyde reductase by aldehydes as suggested above, an increased concentration of NE would be expected but certainly not the long lag period. Since the data represent steady-state levels in whole brain homogenates, the effects could be due to changes in NE storage and/or turnover, or a combination of these plus time factors. It would be difficult, if not impossible, therefore, to compare them

with the results of others (Carlsson et al., 1973; Hunt and Majchrowicz, 1974; Pohorecky, 1974; Majchrowicz, 1975; Karoum et al., 1976; Thadani et al., 1976). For example, the data for MHPG-SO$_4$ at 3 hours (fig. 1) do not agree with those presented by Karoum et al. (1976). However, Karoum et al. (1976) measured free and total MHPG whereas our fluorometric procedure was specific for MHPG-SO$_4$. On the other hand, the results in figure 1 are in complete accord with the data reported by Pohorecky (1974) in a study of NE turnover. Incidentally, the increase that occurred in the controls within the 1st hour is probably due to stress (Duritz and Truitt, 1966).

The discussions above are based on data derived from a limited experimental design, i.e., measurements were made primarily 24 hours after the final dose of ethanol, and also included the drug pyrazole. Our own evidence shows that the latter has marked effects of its own, both peripherally and centrally. On the other hand, recent work has shown that although pyrazole does greatly slow ethanol metabolism, its own metabolism is virtually stopped when blood ethanol levels are high (Deis et al., 1977). Under these conditions, at least 75% of the injected pyrazole was excreted unchanged. Clearly, a broader and yet more specific experimental design is needed to determine whether the observations and speculations reported here are of more than heuristic interest in understanding the effects of ethanol on the central nervous system.

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