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PYRAZOLE AS A MODIFIER OF LIVER MICROSOMAL MONOOXYGENASE IN DBA/2N AND AKR/J MICE

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Abstract—Effects of pyrazole on liver microsomal monooxygenase was studied in two inbred strains of mice, DBA/2N (D2) and AKR/J (AKR). A selective effect on microsomal monooxygenase was found. In the D2 mouse pyrazole strongly increases the coumarin 7-hydroxylase (CoH) and 7-ethoxycoumarin O-deethylase (ECDE) activities while on the total cytochrome P-450 (P-450) content and ethylmorphine *N*-demethylase (EMDM) and benzo(*a*)pyrene hydroxylase (AHH) activities the effect is biphasic (increased with lower doses and decreased with higher). For AKR the effect of pyrazole is different from the D2. The increase of CoH and ECDE is weaker and no biphasic effect for the other three parameters can be seen. Instead only a decrease takes place. The optimal dose of pyrazole for the induction of CoH in the D2 mice is 200 mg/kg once a day during three days. The effect of pyrazole is strongest in animals (D2) of 4–10 weeks old. For young animals (2 weeks old) no effect except of a weak decrease in AHH can be seen. Also for old animals the effect is weak. Recovery of the monooxygenase after pyrazole induction takes place in about 120 hr except for the total P-450 content which is still below normal. No sex dependence in the effect of pyrazole on CoH was found.

In our previous work we have found that in the D2 mouse pyrazole has some exceptional properties as an inducer of the liver microsomal monooxygenase since it increases the ECDE* and especially CoH while AHH and EMDM, for example, in addition to the total microsomal P-450 content are decreased [1]. This type of selective effect is not known previously. A direct proof for pyrazole to be an inducer of the monooxygenase, has been obtained recently by our laboratory when a pyrazole inducible cytochrome P-450 isoenzyme was purified from the livers of D2 mice. This protein has a very high affinity and metabolic capacity both for coumarin and 7-ethoxycoumarin [2].

From previous studies with other species it is known that in rabbit and rat pyrazole increases alcohol oxidizing activities and induces the P-450LM3a or P-450j [3, 4] also inducible by ethanol, acetone, imidazole, isoniazid and trichloroethylene [3]. For effects of pyrazole the dose and the administration time seem to be critical as studied with rats [5, 6]. Unlike in case of rats, pyrazole has not been well characterized as an inducer of the monooxygenase in case of mice. This may, however, be relevant particularly since our primary data indicate that the effects are different.

For this purpose we in this study characterize the potency and the age and sex dependency of the induction by pyrazole. Also the recovery of the monooxygenase after pyrazole treatment is studied. A special attention is paid to the difference in the induction between the D2 and the AKR since according to our previous studies a strong induction by pyrazole takes place only in the D2 mice while it is weak or negligible in the AKR [1, 7].

* Abbreviations used: AHH, benzo(*a*)pyrene hydroxylase; CoH, coumarin 7-hydroxylase; ECDE, 7-ethoxycoumarin-O-deethylase; EMDM, ethylmorphine-N-demethylase; NDMA, dimethylnitrosamine-N-demethylase; P-450, cytochrome P-450; D2, inbred mouse strain DBA/2N//Kuo; AKR, inbred mouse strain AKR/J//Kuo.

MATERIALS AND METHODS

Reagents were obtained from following sources: coumarin (Aldrich Co, Milwaukee, WI), pyrazole, 7-ethoxycoumarin, benzo(*a*)pyrene, fluorescamine, NADPH (Sigma Chemical Co, St. Louis, MO) and ethylmorphine (Yliopiston apteekki, Helsinki, Finland). All other reagents were of the highest grade commercially available.

Biological material. Male or female D2 or male AKR mice, 5–8 weeks old, were used in experiments except for the age-dependent effect of pyrazole where the D2 mice were 2–52 weeks old. Mice were obtained from the National Laboratory Animal Center Kuopio and housed in groups of 5–10 during experiments. Pyrazole was given as an i.p. injection in saline once a day three times 200 mg/kg unless otherwise indicated. Control groups received the same amount of saline. After the last injection the animals were killed in the morning. The microsomal fraction was isolated and stored as described previously [8, 9]).

Analytical procedures. The purity of pyrazole was checked as described before [1]. Protein [10], P-450 [11], ECDE [12], CoH [1], AHH [13, 14] and EMDM [15] were determined according to the methods cited.

RESULTS

Figure 1 shows the effect of increasing dose of pyrazole on five parameters of liver microsomal

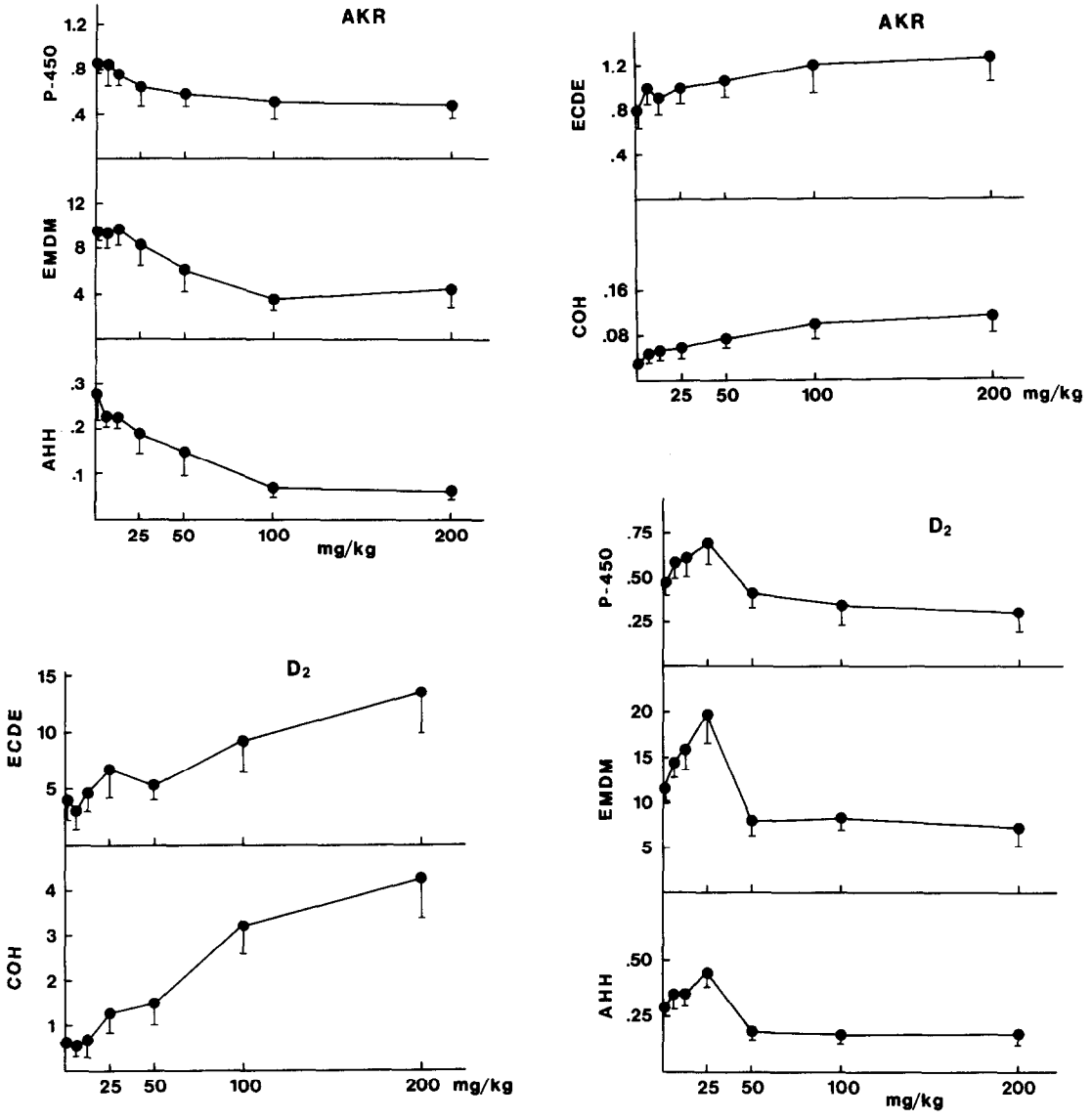

Fig. 1. Dose-dependent modification of microsomal monooxygenase by pyrazole in D2 and AKR mice. The total cytochrome P-450 content is expressed as nmol/mg microsomal protein and the enzyme activities as nmol product formed × min^{-1} × (mg microsomal protein)$^{-1}$. Each observation represents the average ± SD of five animals. For other explanations see abbrevations.

monooxygenase in D2 and AKR mice. A strong dose dependent increase can be seen in the activities of CoH and ECDE in D2 mice. Some increase in these activities also took place for AKR although the effect was weak when compared to D2. It should also be noted that the level of CoH and ECDE in AKR mice was generally much lower than in D2 (Fig. 1).

Response of the three other parameters of the monooxygenase; the total microsomal P-450 and the EMDM and AHH activities on pyrazole treatment was very interesting. In the case of D2 these were increased clearly although not very strongly, with lower doses of pyrazole up to 25 mg/kg, after which pyrazole has a lowering effect as already known from our previous studies [1, 7]. This kind of biphasic effect cannot be seen in the case of AKR since pyrazole even at lower doses decreased the microsomal P-450 content and the EMDM and AHH activities (Fig. 1).

The purpose of the experiment shown in Fig. 2 was to find the dose of pyrazole giving the maximal induction of CoH in D2 mice to be used in further studies. The highest increase in the enzyme activity was reached, with only small variation, with the doses of 2 × 300, 3 × 200 and 3 × 300 mg/kg of which we decided to use 3 × 200 mg/kg for further studies. It should be noted that the 5 days treatment with either 200 or 300 mg/kg does not give any further increase in the enzyme activity. Instead a decrease takes place indicating a toxic effect of the inducing agent.

Figure 3 shows how the age of the D2 mice modifies the effect of pyrazole on the monooxygenase. In general it seems that the effects of pyrazole are

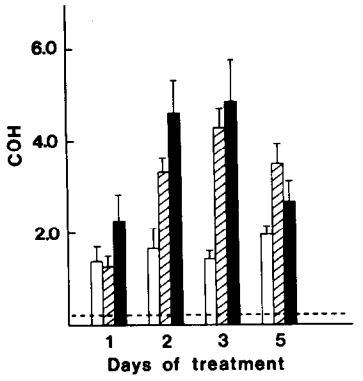

Fig. 2. Effect of pyrazole on microsomal CoH in D2 mice after treatment with 100 (□), 200 (▨) and 300 (■) mg/kg for 1–5 days. Control CoH is indicated by the dotted line through the graph. Each observation represents the average of 3–4 animals. For other explanations see Fig. 1.

strongly age dependent. For the group of the youngest (2 week old) animals pyrazole has no effect except of a weak decrease in the AHH activity. Between 3 and 10 weeks the effects are strong and varying depending on the parameter while for the old (52 weeks) the effects are weaker although still obvious. The results in Fig. 3 are in accordance with those of Fig. 1 where two types of principal effects on the monooxygenase can be seen: a strong increase in ECDE and particularly in CoH and a decrease in EMDM and AHH activities. For EMDM and AHH the profiles are somewhat different which is not surprising in knowing that the main P-450s catalyzing these reactions are different. For the total microsomal P-450 content pyrazole does not seem to have any strong effect. However, there is a general tendency of lowering the P-450 content which for some groups (5 and 7 weeks old) is more clear than for others. For none of the groups the total P-450 content is increased by pyrazole.

Figure 4 demonstrates the recovery of monooxygenase after pyrazole treatment. Again we can see a strong increase in ECDE and CoH activities where maximum values are reached between 12 and 24 hr after the last dose, after which the enzyme activities start decreasing, reaching the control level in about 120 hr. For EMDM and AHH activities the profile of recovery is almost a mirror image of those of ECDE and CoH: a rather strong decrease reaching the maximum in about 24 hr and then increasing back to normal in about 120 hr. For the total microsomal P-450 content the profile is somewhat different: the maximal decrease is reached only after 48 hr and even at 120 hr it is not fully recovered. It is noteworthy that none of the profiles of the enzyme activities follows that of the total P-450 content.

The effect of sex on the pyrazole caused modification in the microsomal monooxygenase as shown in Fig. 5. As can be seen the effect is almost identical both for males and females.

DISCUSSION

Perhaps the most interesting feature of pyrazole as a modifier of the monooxygenase is its selectivity. Although it is well known that certain inducers increase the amount of only certain P-450

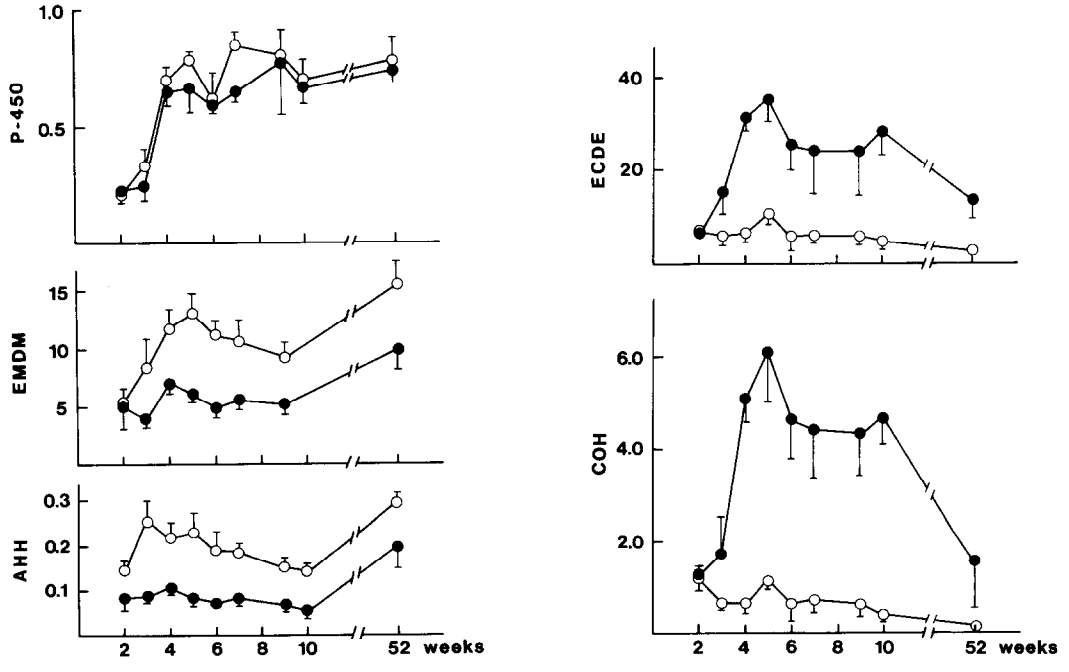

Fig. 3. Effect of age of the D2 mice on the inducibility of microsomal monooxygenase by pyrazole (200 mg/kg once a day during three days). Four control animals (○) and pyrazole treated animals (●) were used for each observation. Other explanations see Fig. 1.

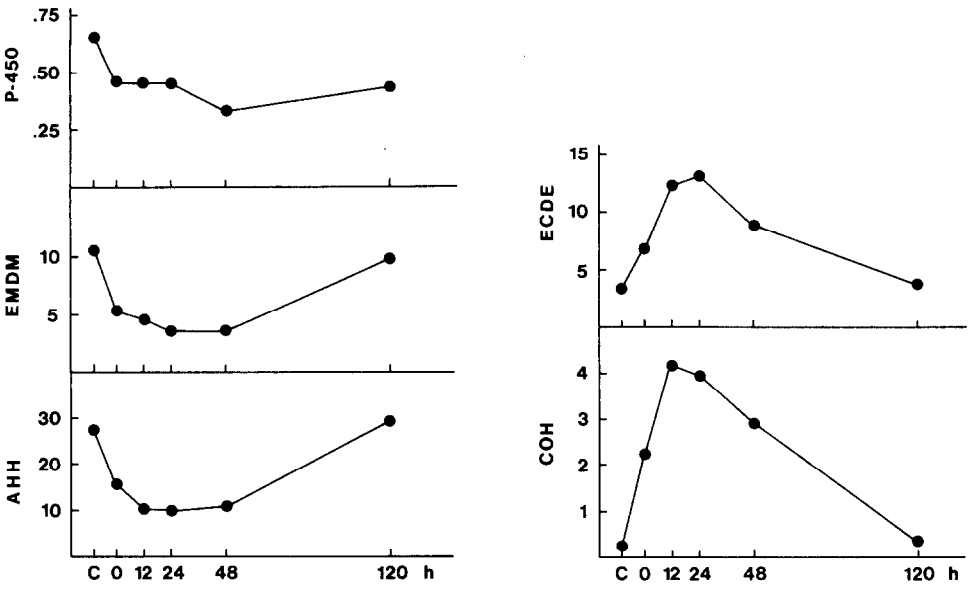

Fig. 4. Recovery of the monooxygenase in D2 mice after treating the animals with 200 mg/kg pyrazole once a day during three days. Each observation is the average of two animals. For other explanations see Fig. 1.

isoenzyme(s) and activities associated with them like methylcholanthrene the P-4501 and the AHH activity [16], phenobarbital the P-450PB-B and dealkylation of pentoxyresorufin [17] and ethanol the P-450LM3a and ethanol oxidation [3], pyrazole has not known previously to be a specific inducer of CoH or any particular P-450 isoenzyme.

On the contrary, CoH has been associated with phenobarbital inducible P-450 system since the activity is in fact increased to some extent by phenobarbital although not nearly as much as with pyrazole [1, 18, 19].

Previous experiments have shown that CoH is under separate genetic control from several other monooxygenase activities [19, 20] and is regulated by the so called CoH-locus [19, 20]. It is thus possible that pyrazole acts as an inducer for this locus specifically. This is further supported by the fact that unlike

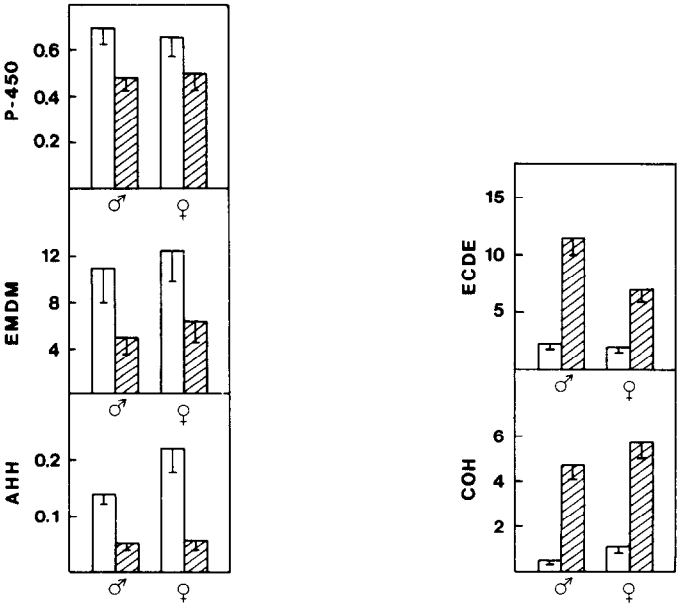

Fig. 5. Sex dependency of the modification of monooxygenase in the D2 mice after pyrazole treatment (200 mg/kg once a day during three days). □, control; ▨, pyrazole treated. For other explanations see Fig. 1.

phenobarbital pyrazole does not increase the total P-450 content or the EMDM activity at all in the AKR and only slightly in the D2 mice.

The second interesting feature of pyrazole as an inducer is its different response on D2 and AKR mice. In the D2 (where the basal CoH and ECDE activities are already higher [7, 18]) the increase of ECDE and CoH is stronger than in the AKR, indicating that the D2 is (more) responsive to pyrazole than the AKR—a situation somewhat analogous to the difference in the AHH induction by the 3-methylcholanthrene in the D2 and B6 mice [21].

Another difference between the AKR and the D2 which may be very important—as far as the induction process is concerned—is the obvious, although only weak, increase in the total P-450 content and EMDM and AHH activities in the D2 totally lacking in the AKR mice. This indicates that in the D2, but not in the AKR, pyrazole acts as an inducer for several P-450 isoenzymes. The fact that with higher doses pyrazole decreases the P-450 content and the EMDM and AHH activities below the control level may be because of its (or its metabolite's) toxicity to the monooxygenase system. This hypothesis is supported by the observations of Craft [5], who has shown that in microsomal suspensions from rats a toxic metabolite is formed from pyrazole destroying the P-450. Whether this destruction process is selective towards different P-450 isoenzymes is not known but theoretically this may be possible. In this case the selectivity of pyrazole is not in the induction but rather in its selective destruction of other P-450s than those metabolizing coumarin or 7-ethoxycoumarin.

Other groups have found pyrazole to be an ethanol-like inducer increasing the amount of P-450LM3a or P-450j in rabbit or rat, respectively [3, 4, 22, 23]. However, several lines of evidence suggest that the P-450 catalyzing the 7-hydroxylation of coumarin and the P-450LM3a and P-450j are different proteins. First, P-450LM3a and P-450j have not been reported to catalyze the metabolism of coumarin. Moreover, CoH is known to be extremely low in the rat [24, 25]. Furthermore, in our recent studies we have found that, as in the case of rabbit or rat, pyrazole increases ethanol oxidation also in mouse liver microsomes. However, this increase is low as compared to the increase in CoH, and takes place in the AKR as well as in the D2. Finally, the pyrazole inducible CoH which we recently purified from the D2 mice is a poor metabolizer of ethanol and also ethanol does not inhibit the CoH catalyzed by this protein.

As an inducer, pyrazole seems to be rather weak since as much as 200 mg/kg is needed for the maximal effect, which is several orders of magnitude more than for TCDD [26, 27] or TCPOBOP [28] which are regarded as the most potent 3-methylcholanthrene- or phenobarbital-type inducers, respectively. The optimal dose of pyrazole is slightly more than for phenobarbital [29]. Also the recovery of the monooxygenase after stopping the pyrazole treatment seems to be rather fast as compared to TCDD or TCPOBOP. This may be due to the fact that pyrazole is rather water soluble and its half life is short [30].

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