# TYROSINE AMINOTRANSFERASE ACTIVITY IN LIVER OF PYRAZOLE-TREATED RATS

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Received 5 February 1976
Revised version received 15 March 1976

## 1. Introduction

We have shown previously [1] that pyrazole administration to rats inhibits the hepatic activity of tryptophan oxygenase [EC 1.13.1.12] probably by reducing the degree of heme saturation of the apoenzyme and thus the enzyme biosynthesis. As pyrazole also affects catalase [EC 1.11.1.6] [2], a specific effect of this compound on hemoprotein enzymes was suggested. However, the data obtained did not allow us, to exclude a more general effect of pyrazole on enzyme synthesis.

It seemed therefore of interest to test whether pyrazole also affects the activity of tyrosine aminotransferase [EC 2.6.1.5], enzyme which, although requiring pyridoxal phosphate and not heme, has, like tryptophan oxygenase, a rapid turn-over in the liver [3] and is also inducible by cortisol [4].

The present results show that pyrazole administration to rats modify the induction time course of tyrosine aminotransferase by cortisol in a way which is similar to that described after administration of known inhibitors of protein synthesis [5,6].

## 2. Materials and methods

Female Wistar rats, weighing 150 ± 5 g, maintained on a standard laboratory diet and fasted during the overnight period immediately preceding the experiments were used.

Pyrazole and cortisol 21-acetate were purchased from Sigma (St Louis, USA).

Pyrazole (272 mg per kg body wt.) and/or cortisol 21-acetate (10 mg per rat) were injected intraperitoneally and the animals killed at various times afterwards, as indicated in Results. Control rats received equivalent volumes of saline. Pyrazole and cortisol, when both administered, were injected at the same time.

The rats being sacrified by decapitation, the whole liver was exposed and frozen exactly one minute after decapitation for a better reproducibility of results. Tyrosine aminotransferase was determined according to Rosen et al. [7] and was expressed as micromoles of *p*-hydroxyphenylpyruvate formed per hour and per g liver wet weight.

All results are given as mean values ± S.E.M. and Student's 't' test was used for comparison of mean values.

## 3. Results

The effects of pyrazole administration on the basal tyrosine aminotransferase activity are shown in fig.1. Whereas the enzyme activity is unaltered 4 and 8 h after treatment, a significant increase (70%) appears 18 h after this treatment.

In order to test whether pyrazole affects the level of tyrosine aminotransferase effectors, we used the mixed homogenate technique, as described by Greengard [8]. The results (table 1) show that the enzyme activity experimentally determined in the mixed homogenates is identical to the theoretical value, thus excluding this possibility.

The effects of pyrazole on the induction of tyrosine aminotransferase by cortisol are shown in

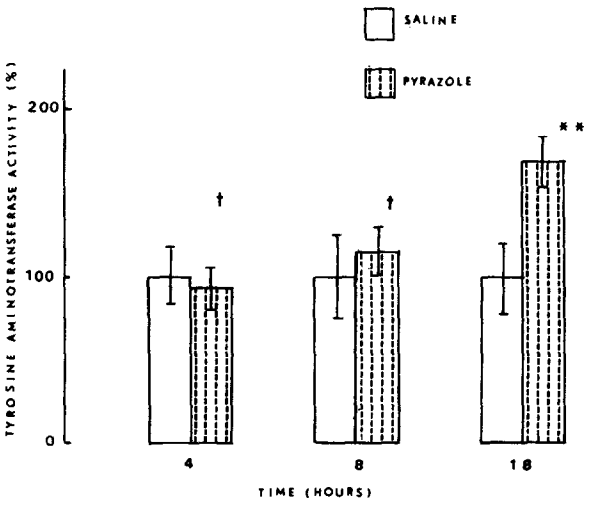


Fig.1. Effects of pyrazole administration on rat liver tyrosine aminotransferase. Pyrazole (272 mg per kg body wt.) was injected at zero time. The enzyme activity is expressed as percentage of the mean activity found at the same time in control saline injected rats. Number of animals: 5 in each group. Statistical significance: † $p > 0.05$, ** $p < 0.01$.

fig.2. The results found 4 h after cortisol administration show that pyrazole does not abolish tyrosine aminotransferase induction by cortisol, although it reduces it by about 20% ($p < 0.01$). 4 h later, tyrosine aminotransferase induced activity does not significantly differ whether or not pyrazole is administered together with cortisol. The results are quite different 10 h later. As a matter of fact, at this time, the tyrosine aminotransferase activity drops back to the basal level in the control animals, treated with cortisol alone, whereas it is maintained at a very high level in the pyrazole plus cortisol treated rats.

As shown by the mixed homogenate technique (table 1), the effects of pyrazole on tyrosine aminotransferase induction are not mediated by changes in effectors of the enzyme activity.

## 4. Discussion

It was found in the present work that, during the first induction phase following cortisol administration,

Table 1
Tyrosine aminotransferase activity 4 and 18 h after pyrazole administration: mixed homogenate assay

| | Single homogenates | | Mixed homogenates | | |
|---|---|---|---|---|---|
| Hours after the treatment | Experimental values | | Experimental values | | Theoretical values |
| Non-induced | A | B | | $\frac{A+B}{2}$ | |
| 4 | 185 ± 34 | 163 ± 20 | 167 ± 10 | $p > 0.20$ | 174 ± 8 |
| 18 | 118 ± 26 | 203 ± 18 | 162 ± 17 | $p > 0.80$ | 160 ± 17 |
| Induced | C | D | | $\frac{C+D}{2}$ | |
| 4 | 527 ± 60 | 424 ± 30 | 488 ± 45 | $p > 0.30$ | 489 ± 42 |
| 18 | 233 ± 55 | 540 ± 69 | 408 ± 69 | $p > 0.60$ | 386 ± 57 |

The animals were given intraperitoneally either saline (A), pyrazole (272 mg per kg body wt.) (B), cortisol (10 mg/rat) and saline (C) or cortisol plus pyrazole (D). The enzyme activities were determined 4 or 18 h after the treatment and are expressed as micromoles of *p*-hydroxyphenylpyruvate formed per hour and per g liver wet weight. They represent the mean ± S.E.M. of 5 rats in each group. The experimental values in the mixed homogenate assays refer to mixtures of equal volumes of homogenates (A) and (B) or (C) and (D).

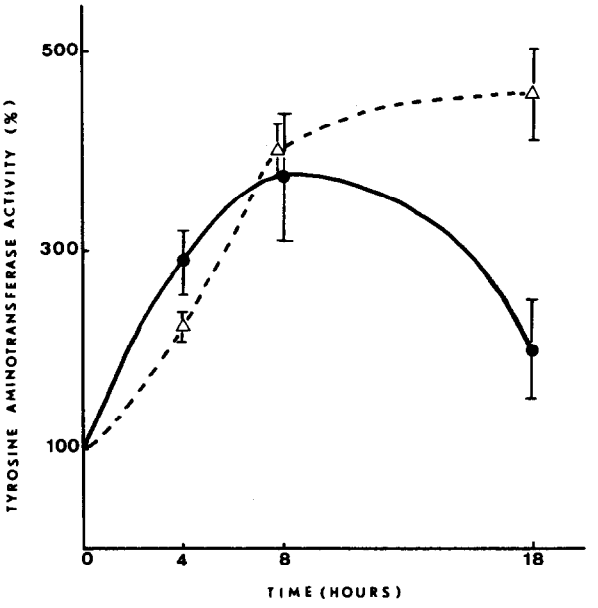


Fig.2. Effects of pyrazole administration on the cortisol mediated induction of rat liver tyrosine aminotransferase. At zero time the animals were given intraperitoneally either cortisol (10 mg/rat) and pyrazole (272 mg per kg body wt.) (- -△- -) or cortisol and saline (—●—). The enzyme activity was determined as described in the text and expressed as percentage of the activity found at the same time in 5 control saline injected rats. Each point represents the mean value ± S.E.M. for 5 rats.

pyrazole reduces by about 20% the activity of tyrosine aminotransferase. This slight inhibition contrasts with the strong reduction (80%) of the tryptophan oxygenase activities found previously [1] during this induction phase, which is characterized by rapid enzyme synthesis [9]. The same discrepancy between the effects on tyrosine aminotransferase and on tryptophan oxygenase has been reported during studies concerned with the effects of inhibitors of protein synthesis such as 8-azaguanine [10,11], 5-fluoroorotate [5] or small doses of cycloheximide [6] on the induction of these enzymes following cortisol administration. The similarity between the results obtained with pyrazole and with known inhibitors of protein synthesis suggests that pyrazole inhibits the enzyme synthesis during the first induction phase, tryptophan oxygenase being much more sensitive to this inhibition than tyrosine aminotransferase.

During the late induction phase (inactivation phase), the activity of tyrosine aminotransferase is maintained at/or near the induced level in the pyrazole plus cortisol treated animals, whereas it drops back to the basal level in the control rats injected with cortisol alone. Similar results have been again reported when studying the effects of 8-azaguanine [11] or 5-fluoroorotate [5] on the late phase of tyrosine aminotransferase induction. The increased basal tyrosine aminotransferase activity found 18 h after pyrazole administration also occurs from 12 to 24 h after cycloheximide treatment [6].

The elevated aminotransferase activity observed 18 h after pyrazole treatment as well as during the late induction phase following pyrazole plus cortisol administration could result from a decreased synthesis either of the repressor acting on the translation of messenger RNA, as suggested by Tomkins et al. [12], or of the polypeptides specifically required for the removal of the enzyme, as suggested by Kenney et al. [13].

Our data suggest that pyrazole administration results in a disturbance of enzyme synthesis which is not restricted to hemoprotein enzymes. This effect should be kept in mind when pyrazole is used in vivo, especially during the course of investigations concerned with alcohol metabolism.

**Acknowledgements**

These investigations were supported by grants from the Institut National de la Santé et de la Recherche Médicale and the Faculté de Médecine de Paris-Ouest.

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