diazepines[19,20]. The presumed pathway from CGP 11952 to **VI** begins with oxidative deamination at the dimethylaminomethyl side-chain, which again yields an aldehyde (fig. 2). This is accompanied by oxidative dealkylation at the ethyl carboxamide side-chain. Formally, the two aldehyde intermediates react with indoxyl (**I**) by loss of one molecule of water. It is probable, however, that the aldehyde intermediates are present as derivatives, e.g. as Schiff-bases with biogenic amines.

Quantitative determination of **VI** in the urine of dogs dosed with [^{14}C] CGP 11952 provided some insight into the mechanism of the above reaction. In urine samples which were stored for a few hours at room temperature, **VI** was readily detected by reverse isotope dilution analysis. In freshly voided urine, collected at 0 °C, **VI** was below the detection limit. This suggests that **VI** and **VIII** are formed only after their aldehyde precursors have passed the kidneys. The reaction seems to be spontaneous rather than enzymic. Aldehydes do indeed react with indoxyl or indoxyl conjugates. A standard method for the measurement of urinary indoxyl is based on its reactions with 4-dimethylaminobenzaldehyde[21].

Mammalian urine contains ample amounts of endogenous indoxyl[6,7]. Xenobiotics can be transformed into aldehyde intermediates by various metabolic reactions[19,20,22]. Therefore, indoxylidene derivatives as detected in this study may also be found in other cases.

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0014-4754/85/040476-03$1.50 + 0.20/0

Rate of tryptophan hydroxylation in vivo in brain nuclei of genetically hypertensive rats of the Lyon strain[1]

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Summary. The rate of tryptophan hydroxylation in vivo is unaltered in brain areas of 5, 9 and 21 week-old Lyon genetically Hypertensive (LH) rats as compared to both Lyon Normotensive (LN) and Low Blood Pressure (LL) rats, except for a decrease in the C1 area of the medulla oblongata in 9 week-old animals.
Key words. Genetically hypertensive rats; serotonin synthesis; tryptophan hydroxylase.

Previous work has permitted the simultaneous selection of one strain of genetically hypertensive rats and of two control strains, which are denominated respectively Lyon Hypertensive (LH), Normotensive (LN) and Low Blood Pressure (LL) strains[2]. As there is evidence that central serotonergic neurons are involved in the control of blood pressure[3], it seemed interesting to determine whether they are functionally altered in this model (LH rats). In the present work, the rate of tryptophan hydroxylation in vivo, which can be considered as a biochemical index of the activity of serotonergic neurons[4], was estimated[5] in discrete brain nuclei of male LH, LN and LL rats. The animals were studied at three ages which characterize the evolution of the blood pressure in these strains, i.e. at 5 and 9 weeks (which correspond respectively to the beginning and to the end of the onset of the hypertension) and at 21 weeks when the hypertension is established[2].

The rate of tryptophan hydroxylation in vivo was estimated by measuring the accumulated 5-hydroxytryptophan (5-HTP) following the pharmacological blockade of the aromatic L-amino acid decarboxylase by NSD 1015[6]. The rats were sacrificed 30 min after the administration of NSD 1015 (50 mg/kg i.p.) and the brains were quickly removed. The following brain areas were dissected out[7]: a) three regions containing serotonin cell bodies: the nuclei raphe centralis, dorsalis and magnus[8], and b) four regions containing serotonin terminals: the medullary C1 and C2 adrenergic regions[9] and the anterior and posterior parts of the hypothalamus. The brain samples were homogenized and after centrifugation the supernatants were used for the 5-HTP determination which was carried out by a sensitive radioenzymatic assay[5]. The proteins were determined in the pellet[10]. As LH, LN and LL rats of the same age were simultaneously killed and assayed, they can be compared safely. No attempt has

Rate of tryptophan hydroxylation in brain nuclei of Lyon Hypertensive (LH), Normotensive (LN) and Low Blood Pressure (LL) rats

Age	5 weeks		9 weeks		21 weeks	
C_1 area						
LH	1.73 ± 0.12*	(9)	1.09 ± 0.15**	(10)	2.32 ± 0.14	(9)
LN	2.47 ± 0.29**	(10)	1.57 ± 0.20	(11)	2.44 ± 0.24	(9)
LL	1.60 ± 0.14	(10)	1.68 ± 0.17	(11)	2.34 ± 0.21	(9)
C_2 area						
LH	2.52 ± 0.32	(9)	3.64 ± 0.32	(10)	3.62 ± 0.32	(9)
LN	2.19 ± 0.31	(10)	3.63 ± 0.32	(11)	3.07 ± 0.32	(9)
LL	2.17 ± 0.26	(10)	3.13 ± 0.28	(11)	3.39 ± 0.41	(9)
Raphe dorsalis						
LH	30.57 ± 3.54	(9)	46.06 ± 4.05	(10)	33.28 ± 3.78	(9)
LN	24.03 ± 2.86	(10)	45.79 ± 3.31	(10)	29.57 ± 3.90	(9)
LL	30.82 ± 2.90	(10)	44.46 ± 2.73	(11)	31.46 ± 3.07	(9)
Raphe centralis						
LH	33.19 ± 3.61	(9)	19.45 ± 3.56	(10)	13.11 ± 1.22	(9)
LN	33.90 ± 6.18	(10)	18.53 ± 3.31	(10)	10.08 ± 1.79	(9)
LL	31.87 ± 2.90	(10)	21.59 ± 2.98	(11)	13.78 ± 2.32	(9)
Raphe magnus						
LH	5.90 ± 1.05	(9)	8.94 ± 0.92*	(10)	9.21 ± 1.36	(9)
LN	4.67 ± 0.58	(10)	6.26 ± 0.58	(10)	8.77 ± 1.82	(9)
LL	6.92 ± 1.09	(10)	8.14 ± 1.14	(11)	10.13 ± 2.00	(9)

Data (ng of L-5-HTP formed/30 min per mg of protein) are means ± SEM. The number of animals in each group is in brackets. Statistical differences between animals of the same age are indicated:
* $p < 0.05$ vs LN rats; ** $p < 0.05$ vs LL rats.

been made to discuss the data as a function of aging, since rats of different ages were killed and analyzed in different runs of assays.

As indicated in the table, the rate of tryptophan hydroxylation of the C1 area was found to be decreased in 5 week-old LH rats (-30%, $p < 0.05$) when compared to LN, but not when compared to LL rats; in 9 week-old LH rats, it was found to be decreased as compared to LN (-30%) and also significantly decreased as compared to LL rats (-35%, $p < 0.05$); at 21 weeks of age, there was no difference between the three strains. There was no significant difference in the C2 area, the nuclei raphe dorsalis, centralis and magnus between the LL, LN and LH rats at the three ages studied, except for an increase in the nucleus raphe magnus of 9 week-old LH rats when compared to LN rats only ($+42\%$, $p < 0.05$). Similarly, no difference was found in the posterior and anterior hypothalamus (data not shown), a result which is similar to the data obtained on Japanese spontaneously hypertensive rats[11,12].

In the present work, the rate of tryptophan hydroxylation was estimated in various brain areas of rats from a genetically hypertensive (LH) or from two control (LN and LL) strains. It might be stated that if a difference is found between LH and LN rats, the same modification must also exist between LH and LL rats to be considered as possibly related to the hypertension. This was not the case for the changes found in LH rats in the nucleus raphe magnus at 9 weeks of age and in the C1 area in 5 week-old animals. Therefore it can be assumed that these alterations are unrelated to the high blood pressure of the LH strain.

On the contrary, the rate of tryptophan hydroxylation of the C1 area was decreased in 9 week-old LH, as compared to both LN and LL rats. Therefore, this change might be related to the difference in the blood pressure levels between these strains. However, it remains to be determined whether there is a link between the hypertension and this transient decrease in the rate of tryptophan hydroxylation in the C1 area. Recent results could support such a link, since serotonergic neurons originating from this medullary area are likely to play an important role in vasomotor control[13].

1 Acknowledgment. The authors wish to thank Dr M. F. Belin, Dr J. F. Pujol and Mrs J. Sacquet for their help during this study. This work was supported by the Fondation pour la Recherche Médicale Française and the C.N.R.S.

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0014-4754/85/040478-02$1.50 + 0.20/0

Involvement of reactive oxygen species in the microsomal S-oxidation of thiobenzamide

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Summary. Superoxide dismutase, catalase and methional proved capable of inhibiting the microsomal oxidation of thiobenzamide, which is most probably catalyzed by the flavin-containing monooxygenase. This indicates that excited oxygen species (e.g. $\cdot O_2^-$, H_2O_2, $\cdot OH$) are involved in the catalytic cycle of this enzymatic reaction. CO, which inhibits the cytochrome P-450-dependent oxygen radical formation, had no effect on the oxidation reaction, suggesting that the source of the reactive oxygen species is not the microsomal mixed-function oxidase.

Key words. Superoxide; hydrogen peroxide; hydroxyl radicals; flavin-containing monooxygenase.

The microsomal flavin-containing monooxygenase (MFMO) is an enzyme capable of oxidizing a wide variety of amine- and sulfur-containing xenobiotics[1,2], leading to the formation of S- or N-oxides which, in many cases, are toxic metabolites. Several reports have revealed the participation of activated oxygen species like superoxide ions or hydroxyl radicals in the catalytic cycles of various oxidases and oxygenases, many of which are flavoproteins[3,4]. As oxygen radicals were reported to be involved