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EX VIVO INHIBITION OF RAT BRAIN CYTOCHROME P-450 ACTIVITY BY STIRIPENTOL

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Abstract—Stiripentol is an anti-epileptic drug of novel structure with previously demonstrated strong *in vitro* inhibitory activity on rat cerebral cytochrome P-450 mediated naphthalene hydroxylation [6]. When administered to rats as a single i.p. dose, the drug is presently shown to have the same *in vitro* effect. Maximal inhibition is seen 2 hr after administration, but at this time the brain concentrations of intact drug, although peaking, appear too low (*ca.* 11 μg/g tissue) to account for the intensity of the effect seen *in vitro*. This suggests *in vivo* activation to a metabolic intermediate forming a complex with cerebral cytochrome P-450, which 2 hr after dosing is fully insensitive to stiripentol added to incubates. Restoration of enzymic activity and of sensitivity to added stiripentol occurs progressively and is practically complete 24 hr after dosing.

Over the years, the brain has been progressively recognized as possessing a variety of xenobiotic-metabolizing activities, in particular cytochrome P-450 mediated monooxygenation reactions (for reviews, see Refs. 1 and 2). The physiological reactions mediated by cerebral cytochromes P-450 include cholesterol side-chain cleavage, the aromatization of androgens to estrogens, the hydroxylation of estrogens to catechol estrogens, and a participation in prostaglandin synthesis. Reactions affecting foreign compounds include oxidative desulfuration, aryl hydrocarbon and phenyl hydroxylation, and various *N*- and *O*-dealkylations [1, 3, 4].

In a previous study [5, 6], we used the hydroxylation of naphthalene to 1-naphthol mediated by rat brain 105,000 *g* particulate fractions to investigate some properties of cerebral cytochrome P-450. In particular, we showed that this activity is strongly inhibited by stiripentol added to the incubates. Stiripentol (**I**) is an anti-epileptic drug of novel structure [7] displaying a methylenedioxybenzene moiety. The latter is characteristic of a group of potent inhibitors of cytochrome P-450 which act by a combination of competitive inhibition and ligand complex formation following oxidation to a carbene intermediate [8–11]. The methylene group in stiripentol is easily oxidized [12], suggesting facile activation to a carbene intermediate, and indeed the compound has been shown to inhibit the oxidation of other drugs in patients [13].

The pharmacodynamic properties of stiripentol imply a central mechanism of action. This raises the question of the *in vivo* relevance of previous results demonstrating the *in vitro* inhibitory effect of stiripentol on rat cerebral cytochrome P-450 [6]. To address this question, we now report the results of an *ex vivo* study where the cerebral oxidation of naphthalene was measured *in vitro* at various time intervals following a single i.p. administration of stiripentol to rats. Marked inhibition was indeed found. To further evidence the involvement of stiripentol, the latter was added to some of the cerebral preparations obtained from stiripentol-pretreated animals.

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MATERIAL AND METHODS

When not otherwise indicated, materials and methods were as reported in the previous study [6].

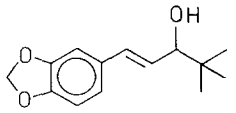
Chemicals. Stiripentol was a gift from Dr. Jacques Astoin (Laboratoire Biocodex, Paris, France).

Animals and preparations of 105,000 *g particulate fractions.* Male Wistar rats weighing 100 g were used. Except stiripentol, they received no pretreatment of any kind. Treated animals received a single i.p. dose of stiripentol (100 mg/kg) in 0.1 ml pure olive oil; control animals received 0.1 ml olive oil. The brains of three to five animals were always homogenized together, and the 105,000 *g* particulate suspension was then placed in the required number of incubation flasks (three to five for each determination). Each time interval thus required three to five treated and three to five untreated rats.

Protein concentrations were measured by the method of Lowry *et al.* [14] for each 105,000 *g* particulate suspension and were usually in the range 3.0–4.0 mg protein/ml.

Incubations. Incubations were carried out at 37° under air for 160 min. Each incubate contained 1 ml of the 105,000 *g* particulate suspension, 3 mg NADPH in 1.5 ml isotonic KCl solution, and 2.5 ml of an isotonic 0.02 M HEPES buffer of pH 7.6. The concentration of naphthalene in the incubates was 10 μg/5 ml (15.6 μM).

Analysis. The extraction and quantitation of 1-naphthol by GC/MS were as previously described



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[6]. For the quantitation of stiripentol in brain preparations, extraction and silylation were as described for 1-naphthol. The retention times of stiripentol and internal standard 1,3-dihydroxynaphthalene (both silylated) were 7.0 and 6.8 min, respectively. Quantitation was done by monitoring the following ions: stiripentol 249, and internal standard 304.2.

RESULTS

Brain concentrations of stiripentol

Following i.p. administration of a single dose of stiripentol, its concentration was measured at various times in both total homogenates and 105,000 *g* particulate fractions. The results (Table 1) show that in both preparations the concentrations are relatively high already 30 min after injection and reach their highest value after 2 hr. For unexplained reasons, the decline appears different in the total homogenate and in the 105,000 *g* particulate fraction. Indeed, the total homogenate loses *ca.* 45% from 2 to 4 hr, then *ca.* 90% from 4 to 8 hr, whereas the particulate fraction loses *ca.* 70% from 2 to 4 hr and then only 40% of the remainder from 4 to 8 hr. A much more detailed distribution and elimination study would be needed to verify and explain, or to reject as unrepresentative these preliminary observations.

Table 1. Brain concentrations of stiripentol in rats following a single i.p. dose of 100 mg/kg (N = 3; ±SD)

Time (hr)	Total homogenate (μg/g tissue)	105,000 *g* particulate fraction (ng/mg proteins)
0	N.D.*	N.D.*
0.5	6.9 ± 2.7	4.2 ± 1.5
1	9.7 ± 1.9	6.5 ± 1.7
2	11.4 ± 6.1	11.0 ± 0.8 (N = 5)
4	6.9 ± 2.7	3.6 ± 0.6
8	0.7 ± 0.2	2.1 ± 0.4

* Not detectable.

Cerebral oxidation of naphthalene in rats pretreated with stiripentol

In these series of experiments, control animals showed activities in the range 3–4 ng 1-naphthol per mg of protein per 160 min. For better readability, however, the results in Table 2 are reported as percentages only. When comparing the data from the first and fourth line in Table 2, it can be seen that naphthalene hydroxylase activity is strongly inhibited in brain preparations from rats sacrificed 2, 4 and 8 hr after stiripentol administration. Two hours after dosing, only 29% of the activity remains, whereas after 4 and 8 hr partial recovery brings the activity to 55–56% of controls (the results after 4 and 8 hr differ significantly from those after 2 hr). After 24 hr, the recovery is apparently complete as no difference exists between treated and untreated animals (the results at 24 hr are significantly different from those after 4 and 8 hr).

It thus appears that inhibition is maximal *ca.* 2 hr after stiripentol administration. This is when the brain concentrations of the intact drug are the highest, namely 11 ng/mg protein (Table 1). This amount corresponds in our incubates to a concentration of approximately 30 nM, which is *ca.* 40 times lower than the IC_{50} value of stiripentol when added directly to the incubates [6]. In other words, the amounts of intact stiripentol remaining in the brain of treated rats are far too low to account for the inhibition seen in these *ex vivo* experiments (see Discussion).

Table 2. Formation of 1-naphthol from naphthalene in brain 105,000 *g* particulate fractions of rats given a single 100 mg/kg i.p. dose of stiripentol at time zero of experiments, and additional inhibitory effects of stiripentol (STI) added to incubates (control values in the range 3–4 ng 1-naphthol per mg proteins per 160 min)

Conditions	Time after i.p. administration (hr) 2	4	8	24
	(results in % of controls ± SD; N = 3–5)			
Control animals (olive oil i.p.)				
no STI added	100 ± 19	100 ± 11	100 ± 24	100 ± 10†
STI added (2 μM)	52 ± 5**	47 ± 11**	39 ± 13*	60 ± 10**
STI added (4 μM)	37 ± 8**	33 ± 5**	41 ± 4*	29 ± 11**
Treated animals (stiripentol i.p.)				
no STI added	29 ± 5**‡§‖	56 ± 12**‡¶	55 ± 17*§††‡‡§§	88 ± 14†‖¶††‖‖¶¶
STI added (2 μM)	29 ± 4**	48 ± 15**	30 ± 4**‡‡	50 ± 23**‖‖
STI added (4 μM)	30 ± 3**	31 ± 14**	26 ± 5**§§	39 ± 22**¶¶

* $P < 0.05$ (relative to 100% controls).
** $P < 0.01$ (relative to 100% controls).
† N = 8.
‡§‖¶†† These horizontal pairs differ with $P < 0.05$.
‡‡§§‖‖¶¶ These vertical pairs differ with $P < 0.05$.

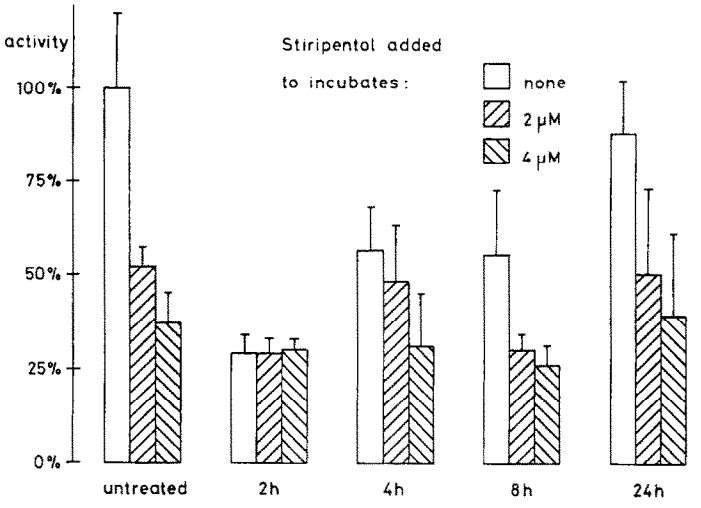

Fig. 1. Inhibitory effect of a single i.p. dose of stiripentol (100 mg/kg) on rat cerebral naphthalene hydroxylase activity at various times after administration, and influence of stiripentol added to incubates.

Effect of stiripentol added to incubates

To investigate further the role of stiripentol, the compound was added at time zero (i.e. together with the substrate) to some of the incubates. When stiripentol was added to preparations from untreated rats (Table 2, second and third lines), inhibitions of *ca.* 50 and 65% were seen for the 2 and 4 μM concentration, respectively, in agreement with previous results [6]. No statistical difference is seen between the 2 and 4 μM concentrations and between the various groups of control animals.

When stiripentol was added to preparations from rats pretreated with the drug, results of a different nature were obtained (Table 2, fifth and sixth lines). With animals killed 2 hr after pretreatment, it is seen that stiripentol added to the incubates has no inhibitory effect *additive* to that due to the pretreatment. Such cerebral preparations are thus fully insensitive to added stiripentol. In contrast, stiripentol is fully active when added to preparations from rats killed 24 hr after pretreatment, implying that such preparations have regained their sensitivity to added stiripentol. Preparations from animals killed 4 and 8 hr after pretreatment yielded intermediate values, indicating a progressive recovery of the sensitivity to stiripentol (note the statistically significant difference within vertical pairs in the third and fourth column). This recovery of the sensitivity to stiripentol paralleled the recovery of hydroxylase activity, as represented schematically in Fig. 1, and confirms the inhibitory effect of stiripentol pretreatment.

DISCUSSION

The results of this study show the rapid penetration and relatively long retention of stiripentol in rat brain. In agreement with *in vitro* findings, a single dose of intraperitoneally administered stiripentol elicits a strong inhibition of cerebral naphthalene monooxygenation as seen in *ex vivo* experiments, and the time course of this effect appears related to the brain concentrations of the drug.

In quantitative terms, however, brain homogenates (with a concentration of *ca.* 10 ng/mg of microsomal protein) supply to incubates amounts of intact stiripentol which yield concentrations of *ca.* 30 nM at maximal inhibition. As explained above, such concentrations are far below the IC_{50} value of stiripentol under these conditions (1.2 μM [6]) and cannot account for the magnitude of the observed inhibitory effect. A likely explanation is *in vivo* cerebral metabolism of the drug to a potent inhibitory metabolite. Indeed, numerous studies [e.g. 8–10] have shown that methylenedioxybenzene derivatives are activated by cytochrome P-450 to a carbene which reacts at the site of formation to form a non-covalent complex with temporary loss of enzymatic activity.

This hypothesis is also compatible with results of experiments where the compound is added to 105,000 *g* preparations from stiripentol-pretreated rats. Two hours after pretreatment, i.e. when inhibition is maximal (*ca.* 70%), *in vitro* added stiripentol is fully devoid of effect, implying (if the above hypothesis is correct) that no additional P-450–carbene complex can be formed. This finding may appear contradictory with results of previous experiments [6] showing that stiripentol 100 μM added to 105,000 *g* fractions from untreated rats results in a practically complete (96.3 ± 2.1%) inhibition of naphthalene oxidation. However, we also showed previously that preincubating cerebral 105,000 *g* preparations with stiripentol 2 μM results in *ca.* 75% inhibition of activity. Taken globally, these results suggest that stiripentol at these concentrations can inhibit (through a postulated inhibitory intermediate) only *ca.* 3/4 of the native rat cerebral cytochrome P-450 mediating naphthalene oxidation. Inhibition of the remaining cytochrome P-450 activity requires very high concentrations (above

10 μM) of stiripentol, which would now act by another, as yet unknown but presumably non-specific, mechanism. This hypothesis is deduced from the known [10] dual mechanism of cytochrome P-450 inhibition displayed by lipophilic methylenedioxybenzenes, namely direct (competitive) and indirect (following activation) inhibition.

Another fact worth noting is that practically full recovery of activity and sensitivity to added stiripentol are seen 24 hr after stiripentol pretreatment. This finding indicates that the P-450–carbene complex, if indeed formed, is not a covalent and irreversible one but that it slowly dissociates within 24 hr to regenerate the functional enzyme. The situation during and following chronic stiripentol treatment can be expected to be more complex, but such studies remain to be undertaken.

In summary, our studies indicate that the novel anti-epileptic drug stiripentol rapidly penetrates into the brain of rats, where it is postulated to be metabolized to an intermediate with strong but transient (less than 24 hr) inhibitory activity towards cerebral cytochrome P-450. The implication of these findings in terms of drug–drug interactions and effects on physiological metabolic pathways remain to be assessed.

REFERENCES

1. Mesnil M, Testa B and Jenner P, Xenobiotic metabolism by brain monooxygenases and other cerebral enzymes. In: *Advances in Drug Research*, Vol. 13 (Ed. Testa B), pp. 95–207. Academic Press, London, 1984.
2. Testa B, Cerebral and other extrahepatic sites of drug metabolism. In: *Development of Drugs and Modern Medicines* (Eds Gorrod JW, Gibson GG and Mitchard M), pp. 288–297. Ellis Horwood, Chichester, U.K., 1986.
3. Walther B, Ghersi-Egea JF, Jayyosi Z, Minn A and Siest G, Ethoxyresorufin *O*-deethylase activity in rat brain subcellular fractions. *Neurosci Lett* **76**: 58–62, 1987.
4. Walther B, Ghersi-Egea JF, Minn A and Siest G, Brain mitochondrial cytochrome P-450sec: spectral and catalytic properties. *Arch Biochem Biophys* **254**: 592–596, 1987.
5. Mesnil M, Testa B and Jenner P, Aryl hydrocarbon hydroxylase in rat brain microsomes. *Biochem Pharmacol* **34**: 435–436, 1985.
6. Mesnil M, Testa B and Jenner P, *In vitro* inhibition of rat brain cytochrome P-450 activity by stiripentol. *Xenobiotica*, in press.
7. Poisson M, Huguet F, Savattier A, Bakri-Longeais F and Narcisse G, A new type of anticonvulsant, stiripentol. Pharmacological profile and neurochemical study. *Arzneim-Forsch (Drug Research)* **34**: 199–204, 1984.
8. Testa B and Jenner P, Inhibitors of cytochrome P-450s and their mechanism of action. *Drug Metab Rev* **12**: 1–117, 1981.
9. Delaforge M, Ioannides C and Parke DV, Ligand-complex formation between cytochromes P-450 and P-448 and methylenedioxyphenyl compounds. *Xenobiotica* **15**: 333–342, 1985.
10. Marcus CB, Murray M and Wilkinson CF, Spectral and inhibitory interactions of methylenedioxyphenyl and related compounds with purified isozymes of cytochrome P-450. *Xenobiotica* **15**: 351–362, 1985.
11. Murray M, Hetnarski K and Wilkinson CF, Selective inhibitory interactions of alkoxymethylenedioxybenzenes towards monooxygenase activity in rat hepatic microsomes. *Xenobiotica* **15**: 369–379, 1985.
12. Moreland TA, Astoin J, Lepage F, Tombret F, Levy RH and Baillie TA, The metabolic fate of stiripentol in man. *Drug Metab Dispos* **14**: 654–662, 1986.
13. Levy RH, Loiseau P, Guyot M, Blehaut HM, Tor J and Moreland TA, Stiripentol kinetics in epilepsy: nonlinearity and interactions. *Clin Pharmacol Ther* **36**: 661–669, 1984.
14. Lowry OH, Rosebrough NJ, Farr AL and Randall RJ, Protein measurement with the Folin phenol reagent. *J Biol Chem* **193**: 265–275, 1951.