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Immunoquantitation of some cytochrome P-450 isozymes in liver microsomes from streptozotocin-diabetic rats

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Summary. Streptozotocin-diabetes in rats leads to a decrease of cytochrome P-450 UT-A (the major form in control rats) and an increase of cytochrome P-450 PB-B (the major one induced by phenobarbital treatment) in liver microsomes. The increased benzphetamine-N-demethylase activity can be related to the induction of cytochrome P-450 PB-B.
Key words. Cytochrome P-450; immunoquantitation; liver microsomes; streptozotocin-diabetes.

Modifications of monooxygenase activities in liver microsomes from streptozotocin (STZ)-diabetic rats have been described in numerous reports[1-4]. Since cytochrome P-450 is a multiprotein family[5-6], these modifications of enzyme activities in STZ-diabetic rats result from changes in the cytochrome P-450 isozymic pattern. The purpose of this study was to investigate this pattern by means of immunoquantitation of some specific isozymes of cytochrome P-450, and to try to correlate it with the monooxygenase activities exhibited by diabetic rats. This new approach may give further insight into the molecular mechanism which sustains drug metabolism in diabetic rats.

Materials and methods. Male rats of the Sprague-Dawley strain (IFACREDO, France), 2½–3 months old were used. Diabetes was produced by a single intrafemoral injection of STZ (80 mg/kg, freshly dissolved in saline solution, pH 4.5) under light ether anesthesia. Glucosuric animals were killed two weeks later. Microsomal membranes were prepared, and cytochrome P-450 and monooxygenase activities were determined, as previously reported[7]. The major cytochromes P-450 isolated from untreated, phenobarbital (PB), B-naphtoflavone (BNF) or isosafrole (ISF)-treated rats were respectively called cytochrome P-450 UT-A, PB-B, BNF-B and ISF-G[8]. Antibodies were prepared as previously described[7]. Anti-UT-A and anti-PB-B were rendered monospecific as indicated[9]. The immunoquantitation of the cytochrome P-450 isozymes was performed by Western Blots as described by Guengerich et al[10].

Results and discussion. In addition to the increase of both cytochrome P-450 and aniline hydroxylase shown before[1-4], we report the increase of benzphetamine-N-demethylase activity in liver microsomes from STZ-diabetic rats (table 1).

The immunoquantitation technique reveals that diabetes does not affect either cyt.P-450 ISF-G or cyt. P-450 BNF-B but that the disease is associated with depressed levels of cyt. P-450 UT-A (by 85%) and increased levels of cyt. P-450 PB-B (8.5-fold) (table 2).

It has been reported that cyt. P-450 PB-B supports with great specificity the N-demethylation of benzphetamine[7]. Therefore it may be suggested that the increased benzphetamine-N-demethylase activity in microsomes from STZ-diabetic rats is the result of the increased level of cyt. P-450 PB-B. This is further indicated by the fact that identical amounts of anti-cyt. P-450 PB-B immunoglobulins produce quite similar inhibitions of the benzphetamine-N-demethylase activity in microsomes from either STZ or PB-treated rats[11].

Thus, the PB-treatment and the STZ-produced diabetes both affect the content of cyt. P-450 PB-B (which is increased) and of cyt. P-450 UT-A (which is decreased). However, they differ by the intensity of their effects: diabetes depresses the level of cyt. P-450 UT-A more than PB-treatment does, but it is less potent in increasing the level of cyt. P-450 PB-B as compared with results obtained in control and PB-treated rats by Guengerich et al.[8] and in our own laboratory (T. Cresteil et al., submitted for publication). Moreover, unlike PB, which acts directly on cyt. P-450 synthesis, the molecule of streptozotocin by itself is not involved in the modification of cytochrome P-450 content and monooxygenase activities: these modifications are the result of the modified hormonal state[3-12].

Moreover, the total immunoquantitated isozymes in liver microsomes from diabetic rats do not account for the level of cytochrome P-450 assayed by the spectrophotometric method. This means that diabetes promotes the synthesis of specific isozymes which differ from the main ones produced by classical inducers. A cytochrome P-450 with high activity towards aniline has been isolated from alloxan-diabetic rats by Past and Cook[13]; it might be one of these specific cytochromes P-450.

Table 1. Cytochrome P-450 content and monooxygenase activities in liver microsomes from normal and diabetic rats

	*Cytochrome P-450	Aniline hydroxylase	Benzphetamine N-demethylase
Control rats	0.87 ± 0.03	0.53 ± 0.05	2.70 ± 0.63
Diabetic rats	1.20 ± 0.04	1.19 ± 0.08	7.96 ± 1.23

*expressed as nmol/mg of microsomal proteins. Enzyme activities are expressed as nmol of product formed × min^{-1} × $nmol^{-1}$ cyt. P-450. Results: mean ± SE of 4–7 animals.

Table 2. Immunoquantitation of some isozymes of cytochrome P-450 in liver microsomes from normal and diabetic rats

	Cyt. P-450 UT-A (a)	Cyt. P-450 PB-B (b)	Cyt. P-450 ISF-G (a)	Cyt. P-450 BNF-B (a)
Control rats	1.58 ± 0.30	0.011 ± 0.005	0.18 ± 0.02	0.017 ± 0.003
Diabetic rats	0.23 ± 0.01	0.093 ± 0.016	0.11 ± 0.01	0.017 ± 0.009

For control and diabetic animals results are the mean ± SEM of 3 animals (a) or 7 animals (b). Each determination is made in duplicate. Results are expressed as nmol of cyt. P-450 × mg microsomal protein. In liver microsomes from control rats, the total of immunoquantitated isozymes exceeds that assayed by the spectrophotometric method. This is explained by the ability of the immunological method to detect also the corresponding apoprotein of the cytochrome P-450 isozyme assayed[7].

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Intranuclear crystalloids in arcuate nucleus neurons after clomiphene citrate administration

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Summary. The action of high doses of clomiphene citrate on the nuclei of hypothalamic arcuate neurons of male cats has been studied. Clomiphene produces an accumulation of typical crystalloid material in the nuclei. After administration of a protein-synthesis inhibitor, no such material was observed in clomiphene-treated animals.
These ultrastructural features could possibly be due to a more intense protein synthesis in the hypothalamic arcuate neurons.
Key words. Hypothalamus; arcuate nucleus; intranuclear inclusions; clomiphene; male cat.

Nuclear inclusions in neurons have been described since the end of the 19th century[1,9,14,20].
The first ultrastructural description of the so-called 'Intranuclear rodlets' of neurons was in 1964[26]. Subsequently, a number of papers treated this subject in areas other than the central and peripheral nervous system under normal, experimental and pathological conditions[2,4,10,18,19,24,25].
Clomiphene citrate has been successfully used to treat anovulatory states of varied etiology in women[13]. Although its exact mode of action has not been established, the final result seems to be the release of pituitary gonadotrophins, particularly luteinizing hormone (LH), and ovulation as a consequence. The regulation of LH-release is one of the likely functions of the arcuate nucleus (ARC) neurons.
The success of this pharmacological agent on spermatogenesis in infertile males varies considerably. Results seem to be dependent on dose, duration of treatment and previous degree of testicular alteration[8,17].
We have excluded several possible causes of variability by using one species and sex (male cat), one specific substance (clomiphene citrate) and the injection into one (intraperitoneal) site of the animals.
Materials and methods. Twelve male cats (1.5–2.0 kg) were raised in our vivarium and housed under controlled environmental and feeding conditions. The following experimental groups were established:
a) Four cats were given a single i.p. injection of clomiphene citrate (250 mg/kg) dissolved in distilled water. These cats were sacrificed 24 h after the injection.
b) Two cats, treated with clomiphene citrate as described above were given a single i.v. injection of protein synthesis inhibitor, cycloheximide (20 mg/kg), 4 h before killing.
c) Two cats were given only cycloheximide as described above 4 h before killing.
d) Two cats were given a single i.p. injection of distilled water.
e) Two cats received no injections and were used as controls.
The animals were anesthetized with Nembutal (35 mg/kg) and

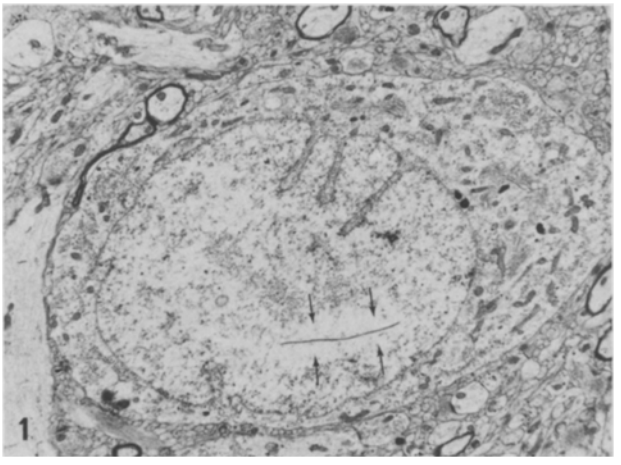

Figure 1. Arcuate nucleus neuron. Crystalloid intranuclear inclusion (arrows) (× 4900).