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Alterations Induced in Distribution and *In Vivo* Metabolism of Imipramine by Pregnenolone-16α-carbonitrile

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Abstract □ Female rats were given pregnenolone-16α-carbonitrile (I) to investigate its *in vivo* effects on the loss of the righting reflex and the mortality rate induced by imipramine as well as the concentrations of this drug and its metabolite, desipramine, in plasma, brain, liver, lungs, and kidneys. The protective action of I was associated with diminished organ levels of imipramine (catatoxic mechanism), and the relationship between brain and plasma imipramine concentrations remained unaltered. Desipramine–imipramine molar ratios were increased, indicating an elevated rate of *N*-demethylation. The unbound imipramine in plasma was diminished, but the relationship between protein-bound and unbound imipramine levels was not modified.

Keyphrases □ Pregnenolone-16α-carbonitrile—effect on pharmacological activity, distribution, and *in vivo* metabolism of imipramine, rats □ Imipramine—effect of pregnenolone-16α-carbonitrile on pharmacological activity, *in vivo* metabolism, and distribution, rats □ Distribution—imipramine, effect of pregnenolone-16α-carbonitrile, rats □ Metabolism—imipramine, effect of pregnenolone-16α-carbonitrile, rats □ Steroids—pregnenolone-16α-carbonitrile, effect on pharmacological activity, distribution, and *in vivo* metabolism of imipramine, rats □ Antidepressants—imipramine, effect of pregnenolone-16α-carbonitrile on pharmacological activity, distribution, and *in vivo* metabolism, rats

The protection afforded by catatoxic steroids against intoxication of diverse etiology, such as analgesics, carcinogens, and pesticides (1), was associated with induction of liver microsomal enzymes, particularly NADPH-dependent mixed function oxidases (2, 3), as well as with decreased levels of these toxicants in plasma, brain, and other tissues (3–7). Pregnenolone-16α-carbonitrile (I), a well-known hepatic microsomal enzyme inducer devoid of any other hormonal or pharmacological properties, exerts the greatest prophylactic effect *in vivo* (1) of the steroids studied.

At present, imipramine is the most frequently used tricyclic antidepressant. Its main metabolic pathways in the body include *N*-demethylation, *N*-oxidation, hydroxylation, and glucuronidation (8–12). Desipramine, one principal metabolite of imipramine, is pharmacologically active (13–15). Both imipramine and desipramine are lipophilic and pass into the brain, their target organ (16).

In view of the importance of imipramine in therapeutics, it was of interest to determine: (*a*) whether a drug-metabolizing enzyme inducer, *e.g.*, I, would produce characteristic alterations in drug distribution; (*b*) whether the changes in plasma drug concentration would be reflected in the brain as well as in the overall pharmacological effect; and (*c*) whether I would modify the influence of plasma protein binding on drug distribution.

EXPERIMENTAL

Female Charles River rats[1], 160–200 g, were maintained on laboratory chow[2] and tap water *ad libitum*. Compound I[3] was given twice daily for 3 days at a dose level of 68.3 mg/kg po in 0.5 ml of water as a micronized suspension homogenized with a trace of polysorbate 80. For comparative purposes, control animals received water with a trace of polysorbate 80. Imipramine hydrochloride[4], 80 mg/kg ip, was administered to all rats in 1 ml of distilled water once on the 4th day, 18 hr after the last pretreatment, unless otherwise stated.

Tables I and II indicate the various time periods at which blood samples were withdrawn under light ether anesthesia by aorta puncture into a syringe containing 0.2 *M* sodium oxalate. Tissues were removed and frozen until required for analysis. Drug-free plasma and tissues were used for preparing standards and blanks.

GLC—Imipramine and desipramine concentrations were measured by GLC[5]. A 1.5-m × 6.4-mm o.d. glass column packed with 1.5% OV-17 on 80–100-mesh Gas Chrom Q was used. The initial column, injector, and detector temperatures were 240, 300, and 300°, respectively. The column temperature was programmed for increases of 32°/min, starting 1 min after the time of injection. The final column temperature (280°) was maintained until 10 min postinjection. The nitrogen flow rate (as the carrier gas) was 18 ml/min, and hydrogen and compressed air pressures

[1] Canadian Breeding Farms & Laboratories Ltd., St. Constant, Quebec, Canada.

[2] Purina Laboratory Chow.

[3] The Upjohn Co., Kalamazoo, Mich.

[4] Ciba-Geigy (Canada) Ltd., Dorval, Quebec, Canada.

[5] Pye Unicam 104 chromatograph equipped with a flame-ionization detector and a Honeywell Electronik 194 Lab-Test recorder coupled with a disk integrator.

Table I—Effect of I on Imipramine Distribution in Tissues and Plasma and on the Brain–Plasma Ratios

Parameter	Imipramine Concentration, μg/g				
	15 min	30 min	60 min	120 min	240 min
Liver					
Control	130.0 ± 12.3 (6)[a]	85.2 ± 5.1 (7)	70.6 ± 5.4 (7)	69.0 ± 6.0 (7)	45.3 ± 3.0 (8)
Pretreated	71.7 ± 5.3 (8)[b]	40.8 ± 3.0 (11)[b]	37.0 ± 1.5 (10)[b]	36.5 ± 1.1 (8)[b]	27.7 ± 0.9 (8)[b]
Lungs					
Control	136.1 ± 8.9 (7)	81.1 ± 7.6 (8)	80.8 ± 2.3 (8)	76.5 ± 3.7 (9)	47.4 ± 1.9 (7)
Pretreated	64.3 ± 4.1 (9)[b]	45.1 ± 4.2 (9)[b]	51.7 ± 2.8 (8)[b]	50.2 ± 2.8 (8)[b]	49.7 ± 1.5 (7)[c]
Kidneys					
Control	92.4 ± 5.7 (7)	91.0 ± 4.5 (7)	65.7 ± 3.9 (8)	52.4 ± 2.9 (8)	—
Pretreated	63.1 ± 4.7 (6)[b]	47.8 ± 4.2 (7)[b]	39.9 ± 1.7 (7)[b]	36.4 ± 1.4 (7)[b]	—
Brain					
Control	62.4 ± 4.9 (5)	84.9 ± 8.5 (5)	57.5 ± 4.4 (6)	38.1 ± 1.1 (7)	—
Pretreated	37.7 ± 2.9 (6)[b]	41.2 ± 2.2 (6)[b]	31.8 ± 2.5 (6)[b]	25.9 ± 1.5 (8)[b]	—
Plasma					
Control	2.6 ± 0.2 (8)	2.3 ± 0.3 (5)	1.5 ± 0.1 (7)	0.9 ± 0.1 (9)	—
Pretreated	1.6 ± 0.1 (8)[b]	1.2 ± 0.1 (8)[b]	0.8 ± 0.1 (10)[b]	0.5 ± 0.1 (8)[b]	—
Brain–plasma ratio					
Control	24	37	38	42	—
Pretreated	24	34	40	52	—

[a] Figures in parentheses indicate number of animals. [b] $p < 0.005$. [c] $p > 0.05$.

Table II—Effect of I on Desipramine Distribution in Tissues and Plasma after Imipramine Administration

Parameter	Desipramine Concentration, μg/g				
	15 min	30 min	60 min	120 min	240 min
Liver					
Control	115.0 ± 6.7 (7)[a]	104.0 ± 7.8 (6)	114.5 ± 13.9 (7)	127.5 ± 11.0 (5)	133.7 ± 16.2 (5)
Pretreated	197.0 ± 9.9 (7)[b]	205.0 ± 16.3 (8)[b]	194.8 ± 16.1 (8)[b]	210.2 ± 7.9 (8)[b]	198.6 ± 16.0 (5)[c]
Kidney					
Control	84.2 ± 6.6 (5)	88.8 ± 7.4 (7)	90.5 ± 5.0 (7)	110.5 ± 7.9 (7)	—
Pretreated	89.5 ± 8.2 (7)[d]	121.1 ± 6.1 (7)[b]	117.9 ± 3.0 (7)[b]	105.2 ± 6.3 (7)[d]	—
Lung					
Control	144.0 ± 11.9 (9)	158.1 ± 7.8 (9)	172.6 ± 11.2 (7)	171.0 ± 13.1 (8)	170.1 ± 9.2 (6)
Pretreated	224.0 ± 16.4 (7)[b]	197.1 ± 8.9 (5)[e]	214.5 ± 12.2 (8)[c]	161.0 ± 4.9 (6)[d]	198.8 ± 7.4 (7)[c]
Brain					
Control	—	17.6 ± 1.2 (5)	—	—	17.8 ± 1.3 (7)
Pretreated	—	38.0 ± 1.6 (5)[b]	—	—	15.3 ± 0.4 (8)[d]
Plasma					
Control	—	3.5 ± 0.5 (4)	—	—	—
Pretreated	—	6.6 ± 0.9 (4)[b]	—	—	—

[a] Figures in parentheses indicate number of animals. [b] $p < 0.005$. [c] $p < 0.05$. [d] $p > 0.05$. [e] $p < 0.01$.

were adjusted to maximize the detector response (hydrogen, 1.2 kg/cm^2; and air, 0.95 kg/cm^2).

Plasma Protein Binding—Free and protein-bound imipramine were measured by a modified *in vivo* dialysis method (17, 18). A cellulose sac[6] containing 4 ml of a 6% dextran[7] solution was implanted subcutaneously, under light ether anesthesia and sterile conditions, on the back through a small incision in the sacral region 24 hr prior to imipramine administration (as described). The sac was removed after 30 min; the contents, pooled from three animals, were analyzed for imipramine (corresponding to the level of free drug).

Measurement of Imipramine and Desipramine—In these studies, imipramine and desipramine were extracted from the biological materials as follows. Organs were homogenized in three parts of water. Aliquots of the resulting homogenate or plasma were pipetted into glass-stoppered centrifuge tubes containing 0.1 ml of 2 *N* NaOH and 0.1 ml of internal standard (3 μg of promazine); the total volume was adjusted to 4 ml with distilled water. In the plasma protein binding experiment, 12 ml of 6% dextran solution, 0.1 ml of 2 *N* NaOH, and 0.1 ml of internal standard (2 μg of promazine) were mixed to give a total volume of 12.2 ml. Heptane (10 ml) containing 1% isoamyl alcohol was added, and the whole mixture was shaken for 10 min.

After centrifugation, 9.5 ml of the organic layer was removed, and the procedure was repeated twice using 5 ml of organic solvent. The combined organic phase was transferred to a tube containing 15 ml of 0.1 *N* HCl, and the tube was shaken for 10 min. After centrifugation, 14.5 ml of the acidic phase was adjusted to pH 11 with 2 *N* NaOH. Freshly distilled ether (8 ml) was added, and the tube was shaken for 10 min and then recentrifuged. As much as possible of the ether layer was removed, dried over anhydrous potassium carbonate, and transferred to glass tubes with finely tapered tips. The ether was evaporated in a water bath at 40°. The tubes were stoppered and cooled in an ice bath to give a final volume of ~0.05 ml of ether/vial, of which approximately 5 μl was injected into the gas chromatograph.

The ratio of peak areas of imipramine or desipramine to the internal standard (corrected for attenuation) was then calculated, and the concentration of each sample was obtained by reference to a standard calibration curve constructed of known amounts of imipramine or desipramine and promazine. The peak area ratios of either substrate to that of promazine were plotted against the concentration of imipramine or desipramine in micrograms per milliliter of plasma or micrograms per gram of tissue; a least mean squares linear regression was performed on the points to get the best-fit straight line.

RESULTS AND DISCUSSION

Acute intoxication with imipramine, measured on the basis of the loss of the righting reflex and the mortality rate at 30 min postinjection (120 mg/kg ip), was strikingly diminished by I. The loss of righting reflex values (positive/total) were: control, 15/15; and I, 1/15. The mortality rates (dead/total) were: control, 15/15; and I, 0/15. After reducing the imipramine dose to 80 mg/kg, the mortality rates were: control, 1/15; and I, 0/15. The loss of righting reflex values were then: control, 15/15; and

Table III—Effect of I on the Desipramine–Imipramine Molar Ratio in Plasma, Brain, and Liver after 30 min

Parameter	Desipramine–Imipramine Molar Ratio		Increase, %
	Control	I Pretreated	
Plasma	1.4	5.2	270
Brain	0.2	0.9	350
Liver	1.2	4.8	300

[6] Diameter, 28 mm; pore radius, approximately 26 Å.
[7] Molecular weight 75,000, Abbott Laboratories, Montreal, Quebec, Canada.

I, 2/15. Convulsions were observed in the controls, while ptosis was evident in all animals.

The decreased pharmacological action correlated with reduced drug concentrations in the brain and other tissues (Table I) at 0.25, 0.5, 1, 2, and 4 hr after imipramine treatment. The plasma drug levels of both groups (control and pretreated) were remarkably low in comparison to the tissue values, with most tissues exhibiting the highest concentrations within 15 min after administration. The elevated brain–plasma ratios showed that the nonpolar character of imipramine enhanced its entry through the blood–brain barrier; the brain attained peak concentrations in 30 min. The similarity between the brain–plasma ratios of control and pretreated animals indicates that I did not modify the permeability of the barrier.

At 15 min postinjection, among all concentrations measured, those of the liver and lung were the highest. However, after 30 min, these differences disappeared and all tissues examined had similar drug concentrations. This result could be attributed to a redistribution of the drug with time. Since the liver is the principal site of detoxication, its high drug levels were not unusual. Persistently elevated concentrations in the lung also were observed in fatal intoxications among humans (19).

Table II summarizes the desipramine values in rats after imipramine administration. Like the parent compound, its plasma concentrations were low compared to those of the tissues after 30 min, indicating a rapid tissue uptake. There was no decline of desipramine levels in the various tissues of I-pretreated and control groups at 0.25, 0.5, 1, 2, and 4 hr, with the exception of the brain of I-pretreated animals which showed a significant reduction after 4 hr.

The molar concentration ratios of desipramine and imipramine in plasma, brain, and liver after 30 min were indicative of differences in the biotransformation rate between control and pretreated animals (Table III). The antisedative effects of imipramine vis-à-vis the syndrome evoked by reserpine and certain benzoquinolizines (20, 21) may be mediated through its metabolite, desipramine (19, 22). According to Bickel and Brodie (23), the *N*-demethylation of tertiary amines results in a loss of their sedative properties. Judging from the 350% increase in the desipramine–imipramine molar ratio in I-pretreated rats, this fact may account in part for the diminished loss of the righting reflex.

The plasma free drug concentrations in control and I-pretreated animals were 0.053 ± 0.010 and 0.025 ± 0.000 μg/ml, respectively. These data provide information with respect to imipramine uptake at the site of action, *i.e.*, the brain. The significantly lower concentration of imipramine capable of penetrating the blood–brain barrier is reflected in the diminished pharmacological response. Compound I, on the other hand, was not effective in altering the percentage of free drug: 2.1 *versus* 2.2% for controls.

In conclusion, I pretreatment reduced the toxic manifestations of imipramine in rats because it enhanced the biotransformation of this drug, resulting in lower tissue concentrations.

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