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Imipramine and its metabolites in human brain

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A quantitative and qualitative thin-layer chromatographic study of the distribution of imipramine and its metabolites in 13 different regions of the brain in a fatal case of intoxication has been made. A quantitative thin-layer chromatographic method for *in situ* determination of imipramine and desipramine has been developed. The concentration of imipramine ranged from 4–36 μg g^{-1} and of desipramine from 0–8 μg g^{-1} in different parts of the brain. 8 identified and 8 unidentified metabolites were detected in the different parts of the brain. Among the identified were hydroxy-, *N*-oxide, and iminodibenzyl-metabolites. The metabolic pattern found corresponds closely to the pattern found in human heart tissue and in urine from patients given imipramine.

The distribution of imipramine in various brain structures has been studied by autoradiography in mice and rat after [^{14}C]imipramine injections (Cassano & Hansson, 1966; Hopf & Eckert, 1969). Dingell, Sulser & Gillette (1964) have determined imipramine and desipramine by a fluorescence method in different tissues including brain in rats and in a 2½ year old child who had taken a lethal dose of imipramine. Bickel, Brochon & others (1967) have determined by a colorimetric method the concentrations of desipramine in different parts of the brain of a 2 year old girl who was a case of fatal intoxication. Sidiropoulos & Bickel (1971), using semi-quantitative thin-layer chromatography, determined imipramine and some metabolites in a lethal case of intoxication in a 15 months old girl.

No reports about the distribution of imipramine and metabolites in different parts of the human brain have been presented.

We have previously investigated the qualitative and quantitative metabolic pattern of [^{14}C]imipramine in human urine (Christiansen, Gram & others, 1967; Gram, Kofod & others, 1971). We now report a study of the qualitative and quantitative metabolic pattern in different parts of the human brain by thin-layer chromatography. The results have been compared to corresponding results obtained from measurements on heart tissue.

MATERIALS AND METHODS

A 23 year old man committed suicide by ingestion of an unknown amount of imipramine (Tofranil) and was found dead 12–24 h later. Whether the drug had been taken regularly before the suicide was not known. The concentration of imipramine was 39 μg ml^{-1} in blood and 155 μg g^{-1} in liver tissue determined by a colorimetric method (Herrmann 1963). Tissues from various parts of brain were removed with the assistance of a trained neuropathologist.

Reagents: All chemicals were of Merck analytical grade.

Extraction

The brain tissues were weighed and homogenized under nitrogen in 10 ml deionized water on a Ultra Turrax homogenizer (2 × 5 s). The homogenates were adjusted to pH 10 by sodium hydroxide 2 M, extracted twice with 25 ml dichlorethane by shaking 5 min in a mechanical shaker and centrifuging (3 min, 3000 rev min^{-1}). The organic phases were dried with anhydrous sodium sulphate, filtered and evaporated to dryness *in vacuo*. To remove most of the lipids in the extract, the residue was dissolved by heating in methanol to which had been added hydrochloric acid 0·1 M 2–3 drops per 10 ml methanol. The lipids were precipitated by cooling, and the supernatant reduced under nitrogen and again cooled to precipitate more lipid. The rest of the lipids were removed by preparative thin-layer chromatography. The extracts were applied as a band on silica-gel G, 0·5 mm thickness and developed 10 cm in chloroform.

Imipramine and its metabolites remain at the origin and the silica gel was scraped off and eluted by methanol—HCl as mentioned above.

The eluates were evaporated to dryness at 37° under nitrogen and stored at –20°.

Chromatography

The residue was dissolved in 1000 μl of methanol—dichlorethane (1: 2), divided in two equal parts and evaporated to dryness at 37° under nitrogen.

One part was used for quantitative and the other for qualitative thin-layer chromatography (t.l.c.).

Quantitative t.l.c.

Merck Silica gel precoated glass plates 20 × 20 cm were used. Solvent system (Solvent I): Benzene–dioxane–ethanol–ammonium hydroxide 25%, (50:40:5:5); development 12 cm.

The residue was dissolved immediately before application in 100 μl methanol–dichlorethane and 5 and 2 × 5 μl were applied to the plate, using a 5 μl double constriction pipette.

Imipramine and desipramine 200 μg ml^{-1} in methanol–dichlorethane were used as standards, 5 μl and 2 × 5 μl were applied. The sample and standards were applied in duplicates on the same plate.

After development the plate was dried 10 min at 110° under nitrogen cooled to about 40–50° also under nitrogen, and placed for 10 min in a chromatographic jar containing fuming nitric acid. Drying and cooling was in a specially constructed oven. The plate was left 1–2 h at room temperature (20°) and the absorption of the coloured spots on the chromatogram was measured *in situ* at 429 nm on a Vitatron TLD 100 densitometer.

The calculation was made from a standard curve for each plate. The coefficient of variation was 5% for imipramine and desipramine (calculated from 7 different chromatograms each with 6 applications of standard solutions).

The calibration curve was linear in the concentration range used.

Qualitative 2-dimensional t.l.c.

The residue was dissolved in about 10–20 μl methanol–dichlorethane and combined with the rest of the extract from the quantitative determination. The total extracts were submitted to 2-dimensional t.l.c. on Silica-gel G, 0·25–0·30 mm accord-

ing to Stahl (1958): first development 12 cm in Solvent I, drying and cooling under nitrogen; second, 12 cm in ethanol saturated with sodium chloride: acetic acid: water (70:20:5) (Solvent II) drying 15 min at 110°. The plates were sprayed with diazoted *p*-nitroaniline (Vidic, 1957), followed by concentrated hydrochloric acid. A key to the 2-dimensional chromatogram is shown in Fig. 1.

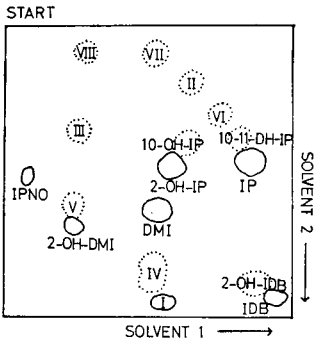

FIG. 1. Key to to the chromatographic pattern. Dotted spots are visible in ultraviolet light (366 nm). See Table 2 for abbreviations.

Table 1. *Distribution of imipramine and desipramine in different parts of the brain and in heart tissue.*

Tissue	g tissue wet weight	$\mu g\ g^{-1}$ Imipramine	$\mu g\ g^{-1}$ Desipramine
Cortex, frontal lobe	7·8	18	7
Cortex, temporal lobe	5·5	18	5
White substance, frontal lobe	3·6	4	1
Thalamus	4·4	25	6
Nucleus caudatus (caput)	4·2	23	6
Nucleus caudatus (corpus)	1·7	28	7
Corpus striatum	3·7	25	8
Mesencephalon	2·4	20	4
Corpora mamillaria	1·3	27	2
Nucleus amygdalae	1·4	36	6
Pons	2·3	13	4
Medulla oblongata (pars distalis)	0·9	20	0
Cortex cerebelli	6·5	5	1
Heart	4·2	27	11

RESULTS

The quantitative results are shown in Table 1. The concentrations of imipramine in different parts of the brain ranged from 4–36 $\mu g\ g^{-1}$ tissue; the lowest concentration was found in the white substance of the frontal lobe and cortex cerebelli and the highest concentration was found in the nucleus amygdalae. The concentration of desipramine was lower in all brain regions and ranged from 0–8 $\mu g\ g^{-1}$ tissue. The concentrations found in heart tissue were for imipramine 27 $\mu g\ g^{-1}$ and for desipramine 11 $\mu g\ g^{-1}$.

The qualitative metabolite patterns in the different parts of the brain are shown in Table 2. 8 identified and 8 unidentified metabolites were found on the chromato-

Table 2. *Distribution of imipramine metabolites in different parts of the brain and in heart tissue, separated by 2-dimensional t.l.c.*

	IP	DMI	IPNO	2-OH-IP	2-OH-DMI	10-OH-IP	10-11-DH-IP	IDB	2-OH-IDB	Unknown I	II	III	IV	V	VI	VII	VIII
Tissue																	
Cortex, temporal lobe	+	+	(+)	+		+	+			+	+	+	+	+	+	+	+
White substance, frontal lobe	+	+				+	+	+					+				
Thalamus	+	+		+		+	+	+			+	+	+	+	+	+	+
Nucleus caudatus (caput)	+	+	+	+		+				+	+	+	+			+	
Nucleus caudatus (corpus)	+	+	+			+	+	+	+	+	+	+	+	+	+		
Corpus striatum	+	+	+	+		+	+	+		+	+	+	+	+	+		
Mesencephalon	+	+	+	+		+	+										
Corpora mamillaria	+	+	+					(+)			+		+		+		
Nucleus amygdalae (Hippocampus)	+	+				+	+	+		+	+		+	+	+		
Pons	+	+		+		+	+	+			+	+	+			+	
Medulla oblongata (pars distalis)	+					+	+	+	+								
Cortex cerebelli	+	+				+	+	+			+	+	+	+			
Heart	+	+		+	+	+				+	+	+	+	+		+	+

Abbreviations: IP = imipramine, DMI = desipramine, 2-OH- = 2-hydroxy-, 10-OH = 10-hydroxy-, IPNO = imipramine-*N*-oxide, 10–11-DH-IP = 10–11-dehydroimipramine, IDB = iminodibenzyl

grams. Desipramine was found in all parts of the brain investigated, except medulla oblongata. Hydroxymetabolites were also found in most parts of the brain, except corpora mamillaria. 10,11-dehydro-imipramine was detected in most parts of the brain except corpora mamillaria and nucleus caudatus (caput). Imipramine *N*-oxide was not found in as many regions as the hydroxymetabolites.

DISCUSSION

The *in situ* quantitative densitometry of substances separated by t.l.c. offers several advantages over conventional methods in drug metabolism studies. The method has a high sensitivity, the parent drug and the metabolites are separated in one procedure and tedious elution techniques are avoided by *in situ* scanning. The difficulty in reproducibility of spraying a reagent on t.l.c. is avoided by the use of vapour technique.

Dingell & others (1964) found in the brain of the 2½ year old child, who died 3½ days after the ingestion of the lethal dose of imipramine, 43 μg g^{-1} imipramine and 35 μg g^{-1} desipramine. Our case—a 23 year old adult—died within 12–24 h of ingesting imipramine. The ratios of imipramine : desipramine that we found are much higher than those found by Dingell & others (1964). This might be a consequence of the time difference in the two cases as man seems to metabolize the two drugs at a limited rate (Dingell & others, 1964). The difference between child and adult must also be taken into consideration. The higher concentration of imipramine than desipramine generally found, which is not so in clinical therapy (Moody, Tait & Todrick, 1967), might indicate that the demethylation process for imipramine is saturated when the body is intoxicated by the drugs.

The particularly high concentration of imipramine we found in nucleus amygdalae, correlates well with the findings of Cassano & Hansson (1969) and Hopf & Eckert (1969) in mice and rat brain. But, our finding of several hydroxymetabolites does not correlate with Bickel & others (1967) who could detect no trace of 2-OH-desipramine in a patient with desipramine intoxication who died approximately 6½ h after ingestion

of the drug. The concentration of desipramine in different parts of the brain ranged from 130–310 $\mu g\ g^{-1}$.

Sidiropoulos & Bickel (1971) found traces of 2-OH-imipramine in the brain of a case of imipramine intoxication 8 days after its ingestion; the concentration of imipramine was 0·600 $\mu g\ g^{-1}$ and of desipramine 1·05 $\mu g\ g^{-1}$.

We found the concentration of imipramine ranged from 4–36 $\mu g\ g^{-1}$ in different parts of the brain, while 2-OH-imipramine, 10-OH-imipramine and 2-OH-iminodibenzyl were detected. 2-OH-desipramine was found only in heart tissue in our study, but this could be due to the difference in the concentration in the tissues. 10,11-dehydro-imipramine was not found in heart tissue but in most parts of the brain.

It is unlikely that the detected metabolites are artifacts, as precautions to avoid oxidation in the analytical procedure were taken.

Most of the metabolites demonstrated in the brain investigated regions have also been found in urine from patients given imipramine (Christiansen & others 1967). It is difficult to find any general rules of the metabolic pattern. The less cell-rich regions, the white substance in the frontal lobes and the medulla oblongata, apparently have smaller amounts of metabolites than other regions. The results indicate that polar substances as hydroxymetabolites either can cross the human blood-brain barrier or be formed in the brain. However results obtained under conditions of acute intoxication do not necessarily correspond to those of long-term therapy.

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