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Blood and brain concentrations of imipramine, clomipramine and their monomethylated metabolites after oral and intramuscular administration in rats

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Imipramine and clomipramine were administered to rats by the oral and intramuscular routes as single and multiple doses. The concentrations of both drugs and their active demethylated metabolites desipramine and desmethylclomipramine were measured in blood plasma, blood cells and brain. The concentrations of the metabolites were higher and the concentrations of the parent substances lower after oral than after parenteral administration, both in blood and in brain. In brain imipramine, desipramine and clomipramine during continuous treatment exceeded their plasma concentrations by six to ten times. The corresponding figure for desmethylclomipramine was 1·7. The extent of accumulation of the investigated substances in the brain was independent of the route of administration.

Measurement of plasma concentrations of imipramine and its active monomethylated metabolite desipramine after a single dose of imipramine hydrochloride to man (Nagy & Johansson, 1975) showed much higher concentrations of metabolite after oral than after parenteral administration as a result of the first pass demethylation of imipramine. We also found preliminarily that the plasma concentration ratio between clomipramine and its monomethylated metabolite desmethylclomipramine was affected by the route of administration. Concentrations of imipramine and desipramine in plasma and brain of animals have been reported by Dingell, Sulser & Gillette (1964) Bickel & Weder (1968) and Jori, Bernardi & others (1971) while Schneider, Schneider & Bickel (1969) observed that different routes of administration influence the metabolism of imipramine. The brain concentrations of clomipramine and its metabolite have not been investigated either after single or multiple doses.

In seeking to find how the route of administration and hence plasma concentrations of the parent substances and their metabolites influence their concentration in the brains of rats, the hydrochlorides of imipramine and clomipramine have been administered by the oral and intramuscular routes as single and multiple doses. The concentration of drug and metabolite were determined simultaneously in the brain, blood plasma and blood cells.

MATERIAL AND METHODS

Animals and administration of the drugs

Male Sprague-Dawley rats, 200–250 g with free access to food and water were given either single doses of 15 mg kg^{-1} of drug or a 10 mg kg^{-1} dose of drug in the morning and again 8 h later on 8 days orally or intramuscularly into the hindleg. The drugs used were commercial solutions for injection containing 12·5 mg of the hydrochlorides of imipramine and clomipramine ml^{-1} (Tofranil and Anafranil, Hässle-Ciba-Geigy AB, Mölndal).

Biological materials

Animals were anaesthestized with ether. After an intracardiac injection of 0·05 ml of heparin solution (5000 unit ml^{-1}, Vitrum, Stockholm) the blood was collected by incision of the heart. The animals were killed (3 at each time) 0·25, 0·5, 1·0, 2·0, 3·5, 5·0, and 7·5 h after the intramuscular and 0·5, 1·0, 2·0, 3·5, 5·0, and 7·5 h after the single oral dose and the brain dissected. During multiple dose treatment animals were killed 8 h after the morning dose. The first determination was made on day 2, i.e. after 3 doses. Blood samples were centrifuged within 30 min and the plasma and cells separated. The brain was weighed, saline solution was added to a final volume of 5 ml and the preparation was homogenized within 30 min. Both plasma and homogenate were stored at −20°.

Analysis of plasma, blood cells and brain homogenate

The drugs and their metabolites were estimated in plasma by thin-layer chromatography and direct densitometry as described previously (Nagy & Treiber, 1973).

For the analysis of blood cells and brain homogenate the extraction procedure was modified as follows. n-Heptane (5 ml) containing 3% amyl alcohol and 2·5 ml of 0·1 M NaOH were added to the sample (5 ml) and the extraction was as for plasma,

repeated twice. The combined organic phases were re-extracted twice with 5 ml of 0·1 M HCl. Two ml of 10 M NaOH was added to the combined aqueous phases which were extracted twice again with n-heptane as above and then chromatographed and quantified as for plasma.

The hydrochlorides of the drugs and metabolites were added as references in different concentrations to plasma, blood cells and brain homogenates from drug-free rats. To obtain a standard curve, five concentrations were analysed together with the samples on each thin-layer plate. The precision of the method at 100 μg litre^{-1} of all the reference substances spiked in pooled plasma samples (n = 10) was in the same range (3–4%). Pooled brain homogenate or blood cell samples (n = 10) at 100 μg litre^{-1} of reference substance gave a precision of 12–15%. Recovery was in the range 70–82%. Spots containing 10–15 ng could be quantified.

RESULTS

Single doses

Fig. 1 shows concentrations against time plots of imipramine and desipramine in the plasma, blood cells and brain after single doses of parent drug administered intramuscularly and orally. The maximum concentration of imipramine was reached after 0·25 h intramuscularly (i.e. in the first sample) both in blood and brain and was eight times higher in brain than in blood. By the oral routes the maximum value was 4 to 5 times less than by the parenteral route but the peak of imipramine in blood and brain occurred at 3·5 h and again brain concentrations were about eight times higher than blood values. The imipramine concentration in blood cells was on average 26% higher than in plasma by both routes.

Only low concentrations of desipramine could be detected in the brain and none in the blood during the first 3 h after parenteral imipramine. The metabolite concentration slowly increased thereafter in both blood and brain and it was about ten times higher in brain than blood. The metabolite appeared in blood and the brain 0·5 h after oral imipramine and reached a peak after 3 h, that in brain exceeding that in plasma by about ten times. The maximum concentration of metabolite was four to five times higher than after parenteral administration. The concentration in blood cells was on average 47% higher than in plasma by both routes.

Fig. 2 shows the results for clomipramine and its metabolite. Intramuscularly, the maximum concentration of clomipramine was reached within 2 h in both plasma and brain and in the brain was about ten times higher than in plasma. Oral administration of the drug produced no detectable concentrations in

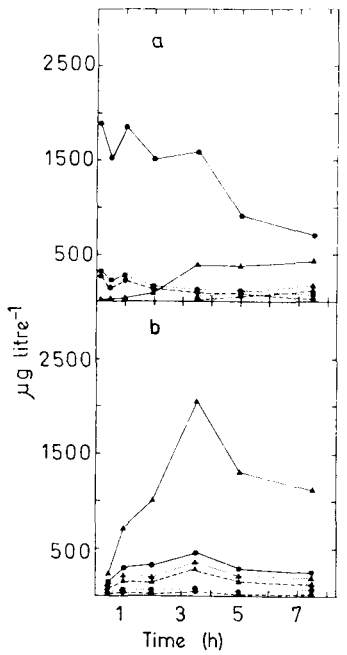

FIG. 1. Plasma (- - - -), blood cell (· · · ·) and brain (—) concentrations of imipramine (●) and desipramine (▲) after a—intramuscular and b—oral administration of single doses of imipramine hydrochloride, (15 mg kg^{-1} body weight).

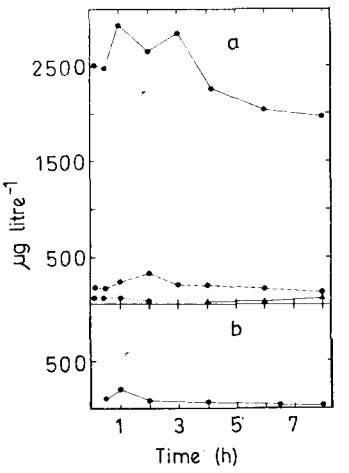

FIG. 2. Plasma (- - - -), blood cell (· · · ·) and brain (—) concentrations of clomipramine (●) and desmethylclomipramine (▲) after a—intramuscular and b—oral administration of single doses of clomipramine hydrochloride, (15 mg kg^{-1} body weight).

blood and those in brain were very low. The highest concentration of the drug in blood cells was in the first sample after intramuscular administration and it was 30% of the corresponding plasma value. Thereafter the concentration rapidly decreased and the drug could not be detected in the cells 3 h later.

Only low concentrations of the metabolite were detected in brain after intramuscular administration and none after oral administration of the parent drug. No metabolite was present in blood with either route.

Multiple doses

Figs 3 and 4 show the concentrations against time of drugs and their metabolites in plasma and brain after continuous intramuscular and oral treatment. Table 1 shows the steady-state levels i.e. the means of the concentrations measured from day 4 to the last day of treatment. With the exception of undetectable blood concentration after oral clomipramine, the parent drugs had reached steady-state levels on day 2, in blood and brain, independently of the route of administration. In contrast, the time for the metabolites to reach steady-state levels differed depending on the route by which the parent drug was given.

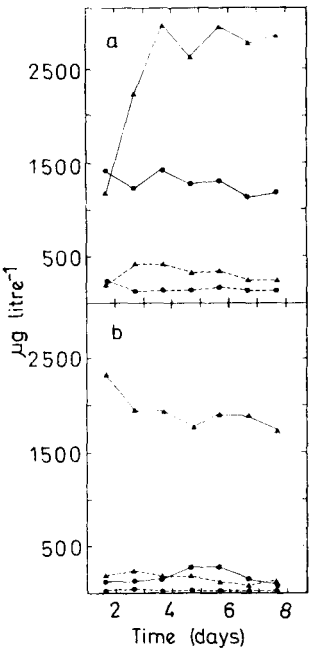

FIG. 3. Plasma (- - -) and brain (—) concentrations of imipramine (●) and desipramine (▲) after a—intramuscular and b—oral administration of 10 mg kg^{-1} body weight of imipramine hydrochloride twice daily for 8 days.

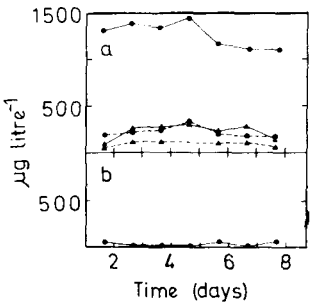

FIG. 4. Plasma (- - -) and brain (—) concentrations of clomipramine (●) and desmethylclomipramine (▲) after a—intramuscular and b—oral administration of 10 mg kg^{-1} body weight of clomipramine hydrochloride twice daily for 8 days.

Desipramine from oral imipramine reached values above the steady-state range in plasma and brain on day 2, i.e. in the first sample, but that formed during intramuscular administration did not reach steady-state levels before day 3–4. A similar tendency was noted for the metabolite when clomipramine was given parenterally.

The ratios between concentrations of parent substances and metabolites in the plasma, blood cells and brain are given in Table 1. The degree of accumulation in the brain tissue was similar for both parent drugs and exceeded their plasma concentration by about six to seven times. The accumulation of the demethylated metabolites was different: in brain concentration of desipramine exceeded the plasma concentration by nine times and the corresponding figure for desmethylclomipramine was 1·7.

DISCUSSION

The results in rats after single doses of imipramine hydrochloride are in agreement with those we found in man (see above). During continuous treatment by different routes, the difference between the steady-state plasma ratio of metabolite to drug did not seem to be so pronounced as the corresponding difference after single doses. Nevertheless, the steady-state ratio was about three to four times higher after oral than after intramuscular treatment in both the plasma and the brain. The higher ratio in the blood cells compared to the plasma is also in agreement with our previous findings (see above).

The low concentrations of clomipramine in brain and its absence in plasma after oral administration indicate low bioavailability. As no metabolite could be detected, poor absorption rather than demethylation would seem to be the reason. These results are not in agreement with our preliminary findings in man and may be valid only for the rat.

Table 1. *Steady-state concentrations of imipramine (IP), desipramine (DMI), clomipramine (CIP) and desmethylclomipramine (DMCI) in the brain, plasma and blood cells after continuous administration of IP and CIP by different routes.*

	IP (10 mg kg^{-1} twice daily)						CIP (10 mg kg^{-1} twice daily)				
Administration	Intramuscular			Oral			Intramuscular			Oral	
Concn*	IP	DMI	DMI/IP	IP	DMI	DMI/IP	CIP	DMCI	DMCI/CIP	CIP	DMCI
Brain (μg kg^{-1})	1287	2790	2·2	215	1820	8·5	1230	232	0·18	44	†
Blood cells (μg litre^{-1})	127	440	3·5	25	217	8·7	9	5		†	†
Plasma (μg litre^{-1})	171	307	1·8	34	173	5·0	208	138	0·66	†	†
Brain/Plasma	7·5	9·1		6·3	10·5		5·9	1·7			

* Average values of determinations on days 4–8. Samples were drawn daily, 8 hours after the last dose.
† Concentration below 5 μg litre^{-1} plasma or 10 μg kg^{-1} brain.

Intramuscularly administered clomipramine seemed to be demethylated to much less extent than imipramine since after single doses only traces of desmethylclomipramine could be detected in the brain and none in the plasma. Continuous intramuscular treatment verified that—assuming that the volume of distribution is not greater for desmethylclomipramine than for desipramine—rats demethylate clomipramine less than imipramine. Furthermore, the desmethylclomipramine formed appeared to be bound to the brain tissue to much less extent than other substances, which showed steady-state concentrations in brain exceeding plasma concentrations by six to ten times. For desmethylclomipramine the figure was 1·7 (Table 1).

Tricyclic antidepressants which are tertiary amines, like imipramine and clomipramine, inhibit the uptake of 5-HT to a greater extent than that of noradrenaline, in contrast to the secondary amines desipramine and desmethylclomipramine, which mainly act on noradrenergic neurons. Clomipramine has been found to be superior to imipramine, and on the whole, to be the most potent inhibitor of 5-HT uptake (Carlsson, Corrodi & others, 1969a, b; Lidbrink, Jonsson & Fuxe, 1971; Ross, Renyi & Ögren, 1972).

It is possible that this effect of clomipramine—demonstrated in rats—is a consequence of the poor ability of the rat to demethylate it and the weak tendency of its metabolite to accumulate in the brain. In the same way, the results for imipramine after *in vivo* treatment may mainly reflect the pharmacological action of the desipramine formed.

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REFERENCES

Bickel, M. H. & Weder, H. J. (1968). *Life Sci.*, **7**, 1223–1230.
Carlsson, A., Corrodi, H., Fuxe, K. & Hökfelt, T. (1969a). *Eur. J. Pharmac.*, **5**, 357–366.
Carlsson, A., Corrodi, H., Fuxe, K. & Hökfelt, T. (1969b). *Ibid.*, **5**, 367–373.
Dingell, J. V., Sulser, F. & Gillette, J. R. (1964). *J. Pharmac. exp. Ther.*, **143**, 14–22.
Jori, A., Bernardi, D., Muscettola, G. & Garattini, S. (1971). *Eur. J. Pharmac.*, **15**, 85–90.
Lidbrink, P., Jonsson, G. & Fuxe, K. (1971). *Neuropharmac.*, **10**, 521–536.
Nagy, A. & Treiber, L. (1973). *J. Pharm. Pharmac.*, **25**, 599–603.
Nagy, A. & Johansson, R. (1975). *Naunyn Schmiedebergs Arch. Pharmac.*, **290**, 145–160.
Ross, S. B., Renyi, A. L. & Ögren, S. O. (1972). *Eur. J. Pharmac.*, **17**, 107–112.
Schneider, G., Schneider, G. & Bickel, M. H. (1969). *Psychopharmacologia (Berl.)*, **16**, 156–160.