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Relative disposition of the GABA agonists THIP and muscimol in the brain of the rat

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Because an alteration of GABA-ergic neurotransmission is believed to be involved in several types of neurological and neuropsychiatric disorders (Roberts 1976), a large variety of drugs acting on GABA-ergic synapses have been developed. Among these drugs one of the most interesting is 4,5,6,7-tetrahydroisoxazolo[5,4-c]-pyridin-3-ol (THIP). THIP belongs to the class of 3-isoxazolol zwitterions (Krogsgaard-Larsen et al 1977). This group of drugs includes muscimol, a weak substrate for GABA reuptake processes and a potent GABA receptor agonist (Krogsgaard-Larsen et al 1975, 1977). THIP is a bicyclic analogue of muscimol with a somewhat weaker intrinsic GABA agonist activity. However THIP, in contrast to muscimol, is not substrate for the GABA reuptake process (Krogsgaard-Larsen et al 1977) and has a low toxicity in various animal species. In acute and chronic toxicological studies THIP is well tolerated by rats, dogs, and baboons (Krogsgaard-Larsen & Christensen 1980).

In the present study the pharmacokinetic properties of [^{3}H]THIP has been studied and compared with those of muscimol. Results indicate that THIP penetrates the blood-brain barrier, distributes unevenly into the rat brain, and is slowly metabolized. Furthermore, both in plasma and brain, the concentration of THIP is several times higher than that of muscimol after systemic administration of the same doses of these compounds.

The present experiments were carried out in male Sprague Dawley rats, ca 150 g (Zivic Miller, Pittsburgh, Pa). The synthesis of [^{3}H]THIP was based upon the incorporation of tritium into a chemically activated derivative of THIP under basic conditions (Krogsgaard-Larsen et al 1982). The synthesized [^{3}H]THIP was purified through BioRad AG 50 × 8 ion exchange column (see Fig. 1). Purified [^{3}H]THIP diluted in 0·4 M $HClO_4$ or added to tissue and then extracted with 0·4 M $HClO_4$ was eluted out of the AG ion exchange column from the third to the fifth ml of NH_4OH (see Fig. 1B). Less than 10% of the radioactive material was eluted from the column in the acid or water fractions (Fig. 1B).

Following intravenous injection of [^{3}H]THIP the radioactive substance (authentic THIP + metabolites) was extracted from plasma and brain with 10 volumes of 0·4 M $HClO_4$. After centrifugation at 30 000 *g* for 20 min, the acid supernatant (2 ml) was applied to AG 50 × 8 column (H^+, 200–400 mesh, 0·5 × 5 cm). The eluate was collected and the column washed with 6 ml of H_2O followed by 7 ml of 2 M NH_4OH (see (Fig. 1A). In the case of both plasma and brain, approximately 90% of the total radioactivity charged to the column was recovered in the eluate. The radioactivity eluted from the column separated into two peaks: the smaller (10–15% of the eluted radioactivity) in the acidic and water fractions and the largest (approximately 80% of the eluted radioactivity) in the NH_4OH fraction. At least 85% of the largest radioactive peak migrated as authentic THIP when analysed by thin layer chromatography (Fig. 2).

The uptake of THIP into different brain areas was measured 30 min following the intravenous injection. The striatum showed the highest concentrations (8·0 ng mg^{-1} tissue) whereas the lowest concentration (4·8 ng mg^{-1} tissue) was detected in the cerebellum (see Table 1). In all brain areas analysed approximately

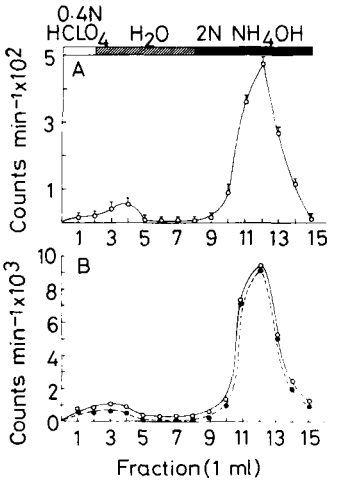

FIG. 1. [^{3}H]THIP elution profile from AG 50 × 8 H^+ column. (A) Two ml of 0·4 N $HClO_4$ brain extract was applied to the column. The extract was obtained from approximately 200 mg brain tissue of 150 g rat injected with 7·5 mg/kg ^{3}H-THIP i.v. The points represent the mean s.e. of four animals. (B) Two ml of standard ^{3}H-THIP in 0·4 M $HClO_4$. ● - - - - ● ^{3}H-THIP; ○ - - - - ○ ^{3}H-THIP added to striatal tissue. As indicated at the top of the figure the column was washed with 6 ml of H_2O followed by 7 ml of 2 M NH_4OH.

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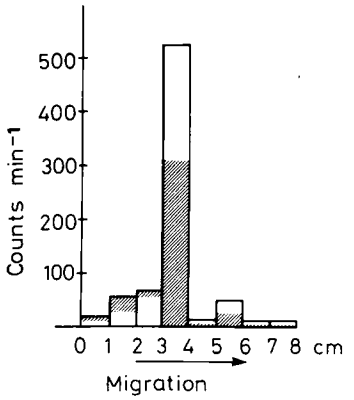

FIG. 2. Identification of [^{3}H]THIP by silica gel 60 high performance thin layer chromatography. The brain extract subjected to AG 50 × 8 purification procedure was applied to a silica gel 60 thin layer plate (from Merck, West Germany). The chromatography was developed with a solvent containing: n-butanol – acetic acid – H_2O (25:4:10). The silica was removed from the plate and was extracted with a mixture of 50% H_2O and 50% ethanol, the extract was measured for radioactivity. □ = Authentic ^{3}H-THIP passed through the AG 50 × 8 purification procedure. ■ = ^{3}H-THIP extracted from brain.

80% of the radioactivity was represented by authentic THIP. This is in contrast to experiments in which [^{3}H]muscimol was injected where the largest amount of radioactivity found in brain (90%) was represented by muscimol metabolites (Baraldi et al 1979).

A comparison of the time course changes of THIP and muscimol in plasma and brain is reported in Table 2. In plasma the concentrations of the two GABA agonists declined steadily during the first hour following the injection. However the half-life of muscimol (≈10 min) was about half that of THIP (≈20 min). In addition, 1 h after an intravenous injection of THIP the plasma concentration of THIP remained constant for 1 h, whereas that of muscimol continued to decline. The net result of these differences in kinetics was that the plasmatic concentration of THIP was several times higher than the concentration of muscimol (Table 2). A similar pattern was observed in the brain: the concentrations of brain THIP were approximately 30 times higher than that of muscimol at 30 min and approximately 100 fold higher at 120 min (Table 2).

Table 1. THIP Brain distribution after intravenous injection of [^{3}H]THIP. Rats (150 g) were injected intravenously with [^{3}H]THIP (11·2 μCi/1·12 mg THIP) and were killed 30 min later. Each value represents the mean (± s.e.m.) of four rats. There was a statistically significant difference among the different brain areas when the values were analysed with the variance analysis test ($F = 3·75$; $F_{4,15,0·05} = 3·06$). Tissues were dissected according to Glowinski & Iversen (1966). The weight in mg wet weight (mean ± s.e.) was: cortex 776 ± 29; striatum 125 ± 8; hippocampus 161 ± 11; cerebellum 291 ± 11.

	[^{3}H]THIP	
Brain region	ng mg^{-1} tissue	μg/brain structure
Cortex	5·8 ± 0·61	4·5
Striatum	8·0 ± 0·75	1·0
Hippocampus	6·8 ± 0·58	1·1
Cerebellum	4·8 ± 0·35	1·4
Remaining brain	6·0 ± 0·70	2·3
Total brain	6·2	10·3

In spite of the large number of pharmacological studies (for review see Krogsgaard-Larsen et al 1981) and the clinical studies in progress (Krogsgaard-Larsen & Christensen 1980), relatively little is known about THIP's ability to penetrate the blood brain barrier, to diffuse into the brain, and to be metabolized. The results of our study indicate that the concentration of authentic THIP measured in brain, 30 min after the administration of 7·5 mg kg^{-1} of [^{3}H]THIP intravenously, is sufficiently high to justify the observation that stimulation of GABA receptors is responsible for its pharmacological actions. This conclusion agrees with the observation that 2 μg of THIP injected into the rat ventricles has clear GABA-mimetic actions such as inducing eating behaviour and producing anticonflict activity (Cananzi et al 1980).

The amount of THIP which penetrates the blood brain barrier following an intravenous injection is 30 and 100 times higher than that of muscimol at 30 and 120 min respectively. This may reflect the higher plasma level of THIP and perhaps in part the higher ability of THIP to penetrate the blood brain barrier (Krogsgaard-Larsen et al 1981). One remarkable difference between muscimol and THIP is that muscimol disappears rapidly from blood. Muscimol is metabolized in large proportions via transamination and its metabol-

Table 2. Time course changes of THIP and muscimol concentrations in plasma and brain of rats. Rats 150 g, were injected intravenously with 1 mg kg^{-1} [^{3}H]THIP (spec.act. 1 μCi mg^{-1}) or 1 mg kg^{-1} [^{3}H]muscimol (spec.act. 100 μCi mg^{-1}). Authentic muscimol was determined as described by Baraldi et al (1979). Each value is the mean ± s.e. of 5 animals.

	Plasma			Brain		
Minutes after injection	μg ml^{-1} THIP	Muscimol	Ratio THIP/Mus	μg g^{-1} THIP	Muscimol	Ratio THIP/Mus
2·5	16 ± 1·2	2·2 ± 0·3	7·2	—	—	—
15	12 ± 0·9	0·76 ± 0·05	15	—	—	—
30	6 ± 0·5	0·30 ± 0·02	20	0·82 ± 0·06	0·024 ± 0·003	34
60	2 ± 0·2	0·06 ± 0·003	33	0·73 ± 0·05	0·018 ± 0·002	40
120	1·7 ± 0·2	0·012 ± 0·002	141	0·77 ± 0·07	0·008 ± 0·001	96

ites penetrate the blood brain barrier (Baraldi 1979; Maggi & Enna 1979). However, THIP is not a substrate for GABA transaminase in vitro (Krogsgaard-Larsen et al 1979). As our study demonstrates, after an initial redistribution THIP disappears very slowly from plasma and few metabolites accumulate in the brain (Table 2). The difference in the pharmacokinetics of THIP and muscimol may very well account for the highest efficiency and lowest toxicity of THIP when this drug is administered systemically.

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The route of administration of imipramine as a factor affecting formation of its metabolite desipramine

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The results of Nagy (1977) and Van Wijk (1977) suggested that the route of administration of the tricyclic antidepressant drug imipramine can significantly modify its metabolism, distribution and pharmacokinetic parameters. After its oral administration the level of its metabolite, desipramine, in rat brain was much higher than that after intramuscular administration of the same dose of drug. To supplement their data, we have made a more detailed study, to find how the route of administration of imipramine and dosage schedule can affect the drug's metabolism and pharmacokinetics. Imipramine's distribution in the rat is similar to that in man, there being a high affinity for brain tissue and low affinity for blood. We have measured the concentration of imipramine and desipramine in blood plasma and brain tissue simultaneously to see if there is a relationship between the plasma and cerebral concentration of the two drugs.

Materials and methods

Male Wistar rats, 180–200 g, used in chronic experiments received standard granulated diet, those in acute experiments were deprived of food for 18 h before use, but had free access to water. Five to seven animals were used for each analytical point.

Imipramine hydrochloride (Tarchomin, Polfa) was given at a dose of 20 mg kg^{-1} intraperitoneally or orally as an aqueous solution. Chronic treatment consisted of daily administration of the dose at the same time of the day, for 14 days.

The animals were decapitated at predetermined times after imipramine, the trunk blood was collected in heparinized tubes and the brains were dissected and stored in solid CO_2 until assayed within 24 h.

The blood and brain levels of imipramine and desipramine were assayed according to Dingell et al (1964). The sensitivity of the method was 0·05 μg/sample for imipramine and 0·07 μg/sample for desipramine, recovery for both compounds was approx. 76%.

Pharmacokinetic parameters $t_{0.5}$, AUC were calculated using a Nonlin-Autoan 2 program (Sedman & Wagner 1976).

The results were evaluated statistically using Student's *t*-test.

Results and discussion

After single i.p. treatment with imipramine the drug reached maximal brain concentration in 35 min, it also disappeared rapidly and after 24 h there was no detectable amount in the c.n.s. The course of accumulation and disappearance of desipramine differed from those of imipramine. The C_{max} appeared 1 h after injection of imipramine, after 24 h there being ca 0·4 μg g^{-1} of desipramine in brain tissue (Fig. 1, Table 1).

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