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Regional brain distribution of risperidone and its active metabolite 9-hydroxy-risperidone in the rat

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Abstract. Risperidone is a new benzisoxazole antipsychotic. 9-Hydroxy-risperidone is the major plasma metabolite of risperidone. The pharmacological properties of 9-hydroxy-risperidone were studied and appeared to be comparable to those of risperidone itself, both in respect of the profile of interactions with various neurotransmitters and its potency, activity, and onset and duration of action. The absorption, plasma levels and regional brain distribution of risperidone, metabolically formed 9-hydroxy-risperidone and total radioactivity were studied in the male Wistar rat after single subcutaneous administration of radiolabelled risperidone at 0.02 mg/kg. Concentrations were determined by HPLC separation, and off-line determination of the radioactivity with liquid scintillation counting. Risperidone was well absorbed. Maximum plasma concentrations were reached at 0.5–1 h after subcutaneous administration. Plasma concentrations of 9-hydroxy-risperidone were higher than those of risperidone from 2 h after dosing. In plasma, the apparent elimination half-life of risperidone was 1.0 h, and mean residence times were 1.5 h for risperidone and 2.5 h for its 9-hydroxy metabolite. Plasma levels of the radioactivity increased dose proportionally between 0.02 and 1.3 mg/kg. Risperidone was rapidly distributed to brain tissues. The elimination of the radioactivity from the frontal cortex and striatum—brain regions with high concentrations of 5-HT_2 or dopamine-D_2 receptors—became more gradual with decreasing dose levels. After a subcutaneous dose of 0.02 mg/kg, the ED_{50} for central 5-HT_2 antagonism in male rats, half-lives in frontal cortex and striatum were 3–4 h for risperidone, whereas mean residence times were 4–6 h for risperidone and about 12 h for 9-hydroxy-risperidone. These half-lives and mean residence times were 3–5 times longer than in plasma and in cerebellum, a region with very low concentrations of 5-HT_2 and D_2 receptors. Frontal cortex and striatum to plasma concentration ratios increased during the experiment. The distribution of 9-hydroxy-risperidone to the different brain regions, including frontal cortex and striatum, was more limited than that of risperidone itself. This indicated that 9-hydroxy-risperidone contributes to the in vivo activity of risperidone, but to a smaller extent than would be predicted from plasma levels. AUCs of both active compounds in frontal cortex and striatum were 10–18 times higher than those in cerebellum. No retention of metabolites other than 9-hydroxy-risperidone was observed in any of the brain regions investigated.

Key words: Risperidone – 9-Hydroxy-risperidone – Active metabolite – Antipsychotic – 5-HT_2 Antagonist – Dopamine-D_2 antagonist – Pharmacokinetics – Regional brain distribution – Rat

Risperidone (R 64766) or 3-{2-[4-(6-fluoro-1,2-benzisoxazol-3-yl)-1-piperidinyl]ethyl}-6,7,8,9-tetrahydro-2 methyl-4*H*-pyrido[1,2-a]pyrimidin-4-one (Fig. 1) is a new antipsychotic drug with potent 5-HT_2 and catecholamine antagonistic properties (Janssen et al. 1988; Leysen et al. 1988, 1992). In clinical studies risperidone was found to improve both the positive and the negative symptoms of schizophrenia, and had a low propensity to induce extrapyramidal side effects (Mesotten et al. 1989; Gelders et al. 1990; Claus et al. 1992). 9-Hydroxy-risperidone (R 76477) is the major plasma metabolite of risperidone in rats and dogs (Meuldermans et al. accepted in 1993) as well as in humans (Mannens et al. 1993). This paper reports on the pharmacological properties of 9-hydroxy-risperidone. Knowledge of the distribution of active compounds to their site of action is important for the interpretation of the relation between pharmacokinetic parameters in plasma and pharmacodynamics (Sunderland and Cohen 1987). It may help to explain why studies in patients often report very modest correlations of blood or plasma levels of neuroleptics with their clinical effects. The time-course of the concentrations of the active moiety at the receptor site might differ considerably from that in whole brain and/or in plasma, especially at therapeutic dose levels. This would imply that risperidone and its active metabolite might persist at the receptor site long after they have been cleared from systemic plasma.

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Besides the pharmacological profile of 9-hydroxy-risperidone, this paper reports on the regional brain distribution of unchanged risperidone, metabolically formed 9-hydroxy-risperidone and total radioactivity in the male Wistar rat, which was studied after subcutaneous administration. To enable evaluation of the in vivo pharmacological profile of risperidone in the rat, the regional brain distribution was studied after a single subcutaneous dose of radiolabelled risperidone. Frontal cortex, striatum and cerebellum were investigated separately. High concentrations of 5-HT_2 receptors are localized in the frontal cortex (Leysen et al. 1982; Pazos et al. 1985) and high concentrations of D_2 receptors in the striatum (Bouthenet et al. 1987). On the other hand, cerebellum was taken as an example of a region with very low 5-HT_2 and D_2 receptor concentrations. In the rest of the brain there were distinct areas that contained 5-HT_2 or D_2 receptors, but they were not further separated.

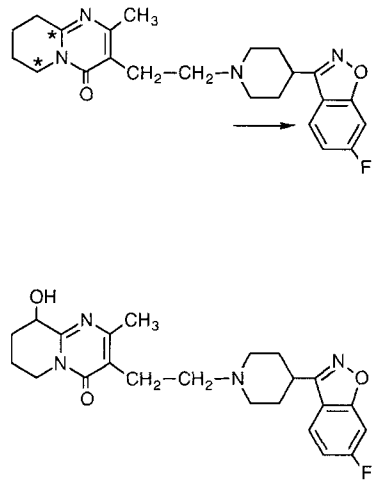

Fig. 1. Structural formula of risperidone and positions of the tritium-label (*arrow*) in ^{3}H-risperidone and of the ^{14}C-labels (*asterisks*) in ^{14}C-risperidone, and structural formula of its active metabolite 9-hydroxy-risperidone

Materials and methods

Pharmacological profile

In vitro receptor binding and neurotransmitter uptake inhibition profile. Inhibition by risperidone and 9-hydroxy-risperidone of radioligand binding to various receptors in tissues and cell membrane preparations, and of the uptake of four neurotransmitters in rat brain synaptosome preparations were investigated as described previously (Leysen et al. 1992).

In vivo tests. Wistar rats and Beagle dogs (Janssen Animal Breeding Centre, Beerse, Belgium) were used. One day before the experiment, animals were transferred to the air-conditioned laboratories (21 ± 2°C, 65 ± 15% relative humidity) and housed in individual cages. Interaction with apomorphine (1.25 mg/kg, IV), tryptamine (40.0 mg/kg, IV), and noradrenaline (1.25 mg/kg, IV) induced behavioural effects was tested successively in the same sets of rats with intervals of 30, 90 and 120 min, respectively, after subcutaneous administration of the test compound as previously described in detail (Niemegeers et al. 1977). It is important to note that in previous studies reporting on the pharmacological profile of risperidone (Janssen et al. 1988; Megens et al. 1992), the three agonists were given to separate sets of animals and different criteria were used for the evaluation of the drug effects; it is evident, in view of the existing interaction between apomorphine and tryptamine (Awouters et al. 1990) that the ED_{50} values found in the present study differ from those reported in previous studies.

Antagonism of apomorphine-induced emesis in dogs (0.31 mg/kg, SC) was tested as described earlier (Niemegeers 1982).

Regional brain distribution

Drug and drug formulations. Specifically ^{14}C-labelled or ^{3}H-labelled risperidone (Fig. 1) were used for these studies. For ^{14}C-risperidone two ^{14}C-labels were incorporated in the 6- and 10-positions of the 6,7,8,9-tetrahydro-2-methyl-4*H*-pyrido[1,2-a]-pyrimidin-4-one moiety, whereas for ^{3}H-risperidone the ^{3}H-label was introduced in the 4-position of the benzisoxazole moiety (Meuldermans et al. in preparation). Specific activities were 412 GBq/mmol (1.00 GBq/mg) for ^{3}H-risperidone and 1.65 GBq/mmol (4.00 MBq/mg) for ^{14}C-risperidone. Radiolabelled risperidone was dissolved in 0.05 M tartaric acid at final concentrations of 0.002, 0.008, 0.032 and 0.13 mg/ml for ^{14}C-risperidone and 0.002 mg/ml for ^{3}H-risperidone. Radiochemical purities of the formulated compounds were ≥ 99% for ^{14}C-risperidone and ≥ 97% for ^{3}H-risperidone.

Animals. Male Wistar rats (Janssen Animal Breeding Centre, Beerse, Belgium), weighing approximately 250 g at dosing time after an overnight fast, were used. Animals were housed individually in stainless steel cages. Tap water was freely available during the experiment. Powdered standard laboratory rat food was available from 4 h after administration.

Experimental design. For the study of the dose-concentration relationship, 16 groups (four groups for each dose) of four rats were dosed with ^{14}C-risperidone formulations by subcutaneous injection into the neck at 1 ml/100 g body weight to provide doses of 0.02, 0.08, 0.32 and 1.3 mg/kg. For each dose, groups of four rats were decapitated at 0.5, 1, 2 and 4 h after administration. From all individual animals, blood was collected on heparin (15–18 IU per ml blood), centrifuged at 2100 *g* for 10 min and plasma was separated. The following brain tissues were dissected separately and weighed immediately in preweighed Combusto-cones (Packard): frontal cortex, striatum (including the nucleus accumbens), cerebellum and rest of brain. Brain samples were stored at 4°C and plasma samples at − 20°C until analysis of total radioactivity (TR).

The 0.02 mg/kg dose was studied more extensively. For this experiment, another eight groups of four rats were dosed with ^{3}H-risperidone by subcutaneous injection into the neck at 1 ml/100 g body weight to provide a dose of 0.02 mg/kg (5 MBq/rat). Groups of four rats were decapitated at 0.25, 0.5, 1, 2, 4, 8, 12 and 24 h after administration. Blood, plasma and brain regions were collected similarly as for the dose proportionality experiment. Tissues were weighed (Table 4), pooled per two rats of the same time group, homogenized and extracted immediately. Samples were stored at − 20°C until analysis.

Radiochemical and analytical methods. For the dose proportionality study, brain tissue samples were freeze-dried (Hetosicc, type CD 13-2) and combusted in a sample oxidizer (Packard, Tri-Carb 306). Carbo-Sorb II (Packard) was used as a CO_2-absorber and Permafluor V (Packard) as a scintillation cocktail. Aliquots of 0.1 ml plasma were diluted with 0.9 ml distilled water and dissolved in 10 ml Instagel II (Packard), used as scintillation cocktail. The radioactivity was counted in individual plasma and tissue samples. For the extensive 0.02 mg/kg study, tissues were homogenized in distilled water (+ 4 ml for frontal cortex and striatum, + 8 ml for cerebellum and + 10 ml for rest of brain), with a Potter-Elvehjem homogenizer (Duall, Kontes). Radioactivity concentrations were measured in individual plasma samples diluted 1:1 (v/v) with distilled water, and in brain homogenates pooled per two rats of the same time interval, either directly (TR), or after lyophilization and

dissolution in 1 ml distilled water. The radioactivity in lyophilized samples was considered as non-volatile radioactivity (NVR). The relative amount of tritiated water was estimated from the difference between the two measurements. Aliquots of 1 ml were dissolved in 10 ml Instagel II. Radioactivity in all samples was measured in a liquid scintillation spectrometer (Packard, model Tri-Carb 2000 CA or Tri-Carb 4530) with automatic conversion of cpm to dpm, and was expressed in ng-equivalents to risperidone.

Plasma and tissue levels of risperidone and 9-hydroxy-risperidone were determined by radio-HPLC with off-line counting of radioactivity. Unlabelled risperidone and 9-hydroxy-risperidone were added in a large excess as internal standards to the samples. Samples were made alkaline (pH ~ 9) with 1 ml 0.05 M sodium borate decahydrate (borax) buffer and extracted twice with 4 ml ethyl acetate. The combined organic layers were back-extracted with 3 ml 0.1 M sulphuric acid. The aqueous phase was made alkaline with 150 μl concentrated ammonia and re-extracted twice with 2 ml ethyl acetate. The combined organic layers were evaporated to dryness, reconstituted in 100-μl aliquots of acetonitrile-distilled water (1:1) and injected on to a Spectra-Physics SP 8700 liquid chromatograph, equipped with a Rheodyne 7125 loop injector and a Spectra-Physics SP 8440 variable-wavelength detector operating at 279 nm. The separation was achieved on a reversed-phase column (15 cm × 2.1 mm I.D.) packed with 5-μm particle-size ODS-Hypersil (Shandon) by a balanced-density slurry procedure with a Haskel DSTV 122-C pump at 7×10^7 Pa. Samples were eluted at ambient temperature with an isocratic solvent system of water-acetonitrile-diethylamine (72:28:0.2). The flow rate was 0.8 ml/min, resulting in retention times of 5 min for 9-hydroxy-risperidone and 8 min for risperidone. Area integration and plotting of the chromatograms was carried out using a Spectra-Physics SP 4100 computing integrator. Effluents of 1.6 ml were collected in counting vials from 0.5 min before, till 1.5 min after the retention times of the compounds to the determined, mixed with 15 ml Instagel II and counted in a liquid scintillation spectrometer. Ultimate sample concentrations were calculated by determination of the ratio of the dpm value and the area of the UV peak and comparison of these ratios with standard curves obtained after analysis of calibration samples prepared by fortification of blank plasma and tissue homogenates with radiolabelled risperidone. Concentrations of 9-hydroxy-risperidone were expressed ng-equivalents to risperidone. Limits of detection for risperidone and 9-hydroxy-risperidone were 0.001 ng(-eq.)/ml plasma, 0.04 ng(-eq.)/g wet tissue for frontal cortex and striatum, 0.03 ng(-eq.)/g wet tissue for cerebellum and 0.005 ng(-eq.)/g wet tissue for the rest of brain.

Data analysis. Data analysis was performed on mean data points. Maximum concentrations (C_{max}) and peak times (T_{max}) were determined by visual inspection. Elimination rate constants (β) were calculated by linear regression of data points (logarithmic concentrations as a function of time) (Gibaldi and Perrier 1982). Elimination half-lives ($t_{1/2,\beta}$) of risperidone were calculated as $\ln 2/\beta$. Areas under the curve (AUCs) were calculated with the trapezoidal rule (Gibaldi and Perrier 1982). AUCs from time t to infinity were calculated by the concentration at time t being divided by β. Mean residence times (MRT) of both the parent drug and its 9-hydroxy metabolite were calculated by dividing the area under the moment curve (AUMC) by the AUC (Gibaldi and Perrier 1982; Chan 1982). For the calculation of concentrations of metabolites other than 9-hydroxy-risperidone [= NVR − (risperidone + 9-hydroxy-risperidone)], non-detected levels of risperidone and 9-hydroxy-risperidone were taken as half the detection limit.

Results

Pharmacological profile of 9-hydroxy-risperidone

In in vitro assays for interaction with specific receptor binding sites and neurotransmitter uptake, the profiles of 9-hydroxy-risperidone and its separate enantiomers (data not shown) were very similar to that of the parent compound risperidone (Table 1). 9-Hydroxy-risperidone and its enantiomers (data not shown) were also very comparable to risperidone in their behavioral interaction with apomorphine, tryptamine, and noradrenaline in rats (Table 2). In dogs, risperidone and 9-hydroxy-risperidone were equipotent antiemetics against apomorphine, displaying a similar onset and duration of action, both along the subcutaneous and the oral route of administration (Table 3).

Regional brain distribution

Risperidone was well absorbed after subcutaneous administration. Plasma levels of radioactivity increased dose proportionally after subcutaneous administration of between 0.02 and 1.3 mg/kg (Fig. 2). After subcutaneous dosing at 0.02 mg/kg, peak plasma levels of NVR were reached within 1 h (Fig. 3). They averaged 5.9 ng-eq./ml (Table 4). Peak levels of unchanged risperidone amounted to 4.2 ng/ml at 0.5 h after administration. At that time risperidone levels represented 87% of the NVR. From 1 h after administration, risperidone was eliminated with an apparent half-life of 1.0 h. In plasma, the mean residence time of the parent compound after subcutaneous administration was 1.5 h. A comparison of AUCs revealed an average risperidone to NVR ratio of 0.35. Concentrations of 9-hydroxy-risperidone accounted for the largest fraction of the plasma metabolites up to 4 h after dosage. Maximum plasma levels of 9-hydroxy-risperidone, reaching 1.9 ng-eq./ml, were observed at 2 h. After peak time, plasma levels of this metabolite declined more gradually than those of the parent drug. The mean residence time of 9-hydroxy-risperidone after subcutaneous administration of risperidone was 2.5 h. A comparison of AUCs revealed an average 9-hydroxy-risperidone to NVR ratio of 0.29. The 9-hydroxy-risperidone to risperidone AUC ratio was 0.83. The formation of tritiated water (THO) was very limited. Plasma concentrations of THO never exceeded 0.1 ng-eq./ml.

After subcutaneous administration, concentrations of the radioactivity in brain initially rapidly increased at all investigated dose levels. In cerebellum and in plasma, concentrations of the radioactivity declined at a similar rate over the whole dose range investigated. The elimination of the radioactivity from frontal cortex and striatum, however, became more gradual with decreasing dose levels. For the latter two brain regions, tissue to plasma concentration ratios increased during the experiment, and this increase was larger with lower doses of risperidone (Fig. 4).

After subcutaneous administration at 0.02 mg/kg, risperidone was rapidly distributed to brain tissues (Fig. 5). In all brain regions investigated, concentrations of risperidone were maximal at 1 h after dosing (Table 4). Highest concentrations of risperidone were reached in frontal cortex and striatum, amounting to 6.9 and 5.8 ng/g. Concentrations of risperidone declined with a half-life in the order of 3.9 h in frontal cortex and 2.5 h in striatum, whereas mean residence times were 5.7 h in

Table 1. Receptor binding and neurotransmitter uptake inhibition profile of risperidone and 9-hydroxy-risperidone

Receptor binding site	Radioligand	Tissue (species, area)	K_i values (nM), mean ± SD (*n*) risperidone		9-hydroxy-risperidone[a]	
Serotonin-5HT$_2$	^{3}H-Ketanserin	Rat, frontal cortex	0.12 ± 0.02	(4)	0.22 ± 0.04	(4)
Serotonin-5HT$_{1C}$	^{3}H-Mesulergine	Pig, choroid plexus	47 ± 6	(3)	48 ± 11	(4)
Serotonin-5HT$_{1D}$	^{3}H-Serotonin	Calf, substantia nigra	52 ± 16	(4)	123 ± 20	(2)
Serotonin-5HT$_{1A}$	^{3}H-8-Hydroxydipropyl-amino-tetralin	Rat, hippocampus	270 ± 40	(3)	400 ± 40	(4)
Serotonin-5HT$_{1B}$	^{3}H-Serotonin	Rat, hippocampus	3700 ± 1.700	(3)	2530 ± 330	(2)
Serotonin-5HT$_3$	^{3}H-GR65630	N × G 108CC15 cells	NA[b]		NA	
α_1-Adrenergic	^{3}H-WB4101	Rat, forebrain	0.81 ± 0.14	(4)	1.3 ± 0.2	(4)
α_2-Adrenergic	^{3}H-Clonidine	Rat, cortex	7.3 ± 1.2	(4)	15 ± 3	(3)
β_1-Adrenergic	^{125}I-Cyanopindolol	Human-β_1 cloned, *E. coli*	NA		NA	
β_2-Adrenergic	^{125}I-Cyanopindolol	Human-β_2 cloned, *E. coli*	NA		NA	
Histamine-H$_1$	^{3}H-Pyrilamine	Guinea pig, cerebellum	2.10 ± 0.01	(3)	7.9 ± 0.5	(3)
Histamine-H$_2$	^{3}H-Tiotidine	Guinea pig, striatum	900 ± 300	(3)	4600 ± 300	(3)
Dopamine-D$_2$	^{3}H-Haloperidol	Rat, striatum	3.0 ± 0.9	(4)	4.1 ± 0.7	(3)
Dopamine-D$_1$	^{3}H-SCH23390	Rat, striatum	620 ± 100	(3)	660 ± 194	(4)
Release site	^{3}H-Ketanserin	Rat, striatum	130 ± 30	(3)	390 ± 120	(4)
Haloperidol-sensitive σ sites	^{3}H-Haloperidol	Guinea pig, medulla oblongata	800 ± 220	(3)	1327 ± 0	(3)
Na^+ channel	^{3}H-Batrachotoxin	Rat, cortex	3850 ± 650	(3)	8000 ± 1800	(3)
Ca^{2+} channel	^{3}H-Nitrendipine	Rat, cortex	NA		NA	
μ-opiate	^{3}H-Sufentanil	Rat, forebrain	NA		NA	
Cholinergic muscarinic	^{3}H-Dexetimide	Rat, striatum	NA		NA	
TCP[c] *N*-methyl-D-aspartate sites	^{3}H-TCP	Rat, hippocampus	NA		NA	
Benzodiazepine	^{3}H-Flunitrazepam	Rat, forebrain	NA		NA	
Cholecystekinin CCK-A	^{3}H-CCK-8 (sulphated), propionylated	Rat, pancreas	NA		NA	
Cholecystekinin CCK-B	^{3}H-CCK-8 (sulphated), propionylated	Guinea pig, total cortex	NA		NA	
Neurotensin	^{3}H-Neurotensin	Guinea pig, forebrain	NA		NA	
Substance P	^{3}H-Substance P	Rat, forebrain	NA		NA	
Leukotriene D$_4$	^{3}H-Leukotriene D$_4$	Guinea pig, lung	NA		NA	
Platelet-activating factor	^{3}H-Platelet-activating factor	Rabbit platelets	NA		NA	
Thromboxane A$_2$	^{3}H-SQ29548	Human platelets	NA		NA	
Neurotransmitter uptake	**Radioligand**	**Tissue (species, area)**	**IC_{50} (nM), mean ± SD (*n*) risperidone**		**9-hydroxy-risperidone[a]**	
Serotonin	^{3}H-Serotonin	Rat, cortex	540 ± 80	(4)	2170 ± 300	(3)
Noradrenaline	^{3}H-Noradrenaline	Rat, hypothalamus	2540 ± 480	(4)	830 ± 150	(3)
Dopamine	^{3}H-Dopamine	Rat, striatum	4740 ± 390	(3)	9800 ± 3700	(3)
γ-Aminobutyric acid	^{3}H-γ-Aminobutyric acid	Rat, cortex	NA		NA	

[a]Values determined for separate enantiomers were similar.
[b]NA = not active at the highest tested concentration of 10^{-5} M.
[c]TCP = *N*-[1-2-thienylcyclohexyl]-3,4-piperidine
Data for risperidone were taken from Leysen et al. (1992)
Tests were ordered according to increasing K_i-values of risperidone; groups of neurotransmitter receptor subtypes were kept together, within each group receptor subtypes were ordered according to increasing K_i-values of risperidone

frontal cortex and 3.8 h in striatum. Frontal cortex and striatum to plasma concentration ratios of risperidone rapidly increased (Fig. 6). Comparison of AUCs revealed average tissue to plasma ratios of 5.1 for frontal cortex and 2.8 for striatum. Concentrations of risperidone in cerebellum reached 1.4 ng/g at 1 h after administration and thereafter declined at a similar rate to corresponding plasma levels. The cerebellum to plasma concentration ratio of risperidone was approximately 0.3. In the rest of brain, the concentration of risperidone was intermediate between that in frontal cortex and striatum on the one hand and that in cerebellum on the other. After a dose of 0.02 mg/kg, a comparison of AUCs revealed a tissue to plasma ratio of 1.8 for risperidone in the rest of brain.

At initial time points, concentrations of 9-hydroxy-risperidone in all brain regions investigated were markedly lower than in plasma (Fig. 5). However, for frontal cortex and striatum, tissue to plasma concentration ratios of 9-hydroxy-risperidone, just as those of risperidone, increased as a function of time (Fig. 6). Maximum

Table 2. Comparison of the pharmacological profile of risperidone and 9-hydroxy-risperidone in the apomorphine (APO), tryptamine (TRY), and noradrenaline (NOR) interaction test in rats (ATN test). Interaction with APO (1.25 mg/kg, IV), TRY (40.0 mg/kg, IV) and NOR (1.25 mg/kg, IV) induced behavioural effects was tested successively in the same sets of rats with intervals of 30, 90 and 120 min, respectively, after subcutaneous administration of the test compound

Pharmacological activities	ED_{50} (mg/kg), mean (95% confidence limits) risperidone	9-hydroxy-risperidone[a]
Antagonism of APO-induced agitation	0.15 (0.11–0.20)	0.39 (0.26–0.58)
Antagonism of APO-induced stereotypy	0.15 (0.11–0.20)	0.39 (0.26–0.58)
Stimulation of APO-induced agitation or stereotypy	> 10	> 10
Decrease of palpebral opening after APO	0.89 (0.59–1.3)	1.8 (1.2–2.7)
Increase of palpebral opening after APO	> 10	> 10
Stimulation of mortality after APO	> 10	> 10
Antagonism of TRY-induced bilateral clonic convulsions	0.13 (0.079–0.21)	0.22 (0.18–0.28)
Antagonism of TRY-induced tremors	0.11 (0.069–0.18)	0.34 (0.18–0.65)
Stimulation of TRY-induced bilateral clonic convulsions or tremors	> 10	> 10
Decrease of palpebral opening after TRY	0.19 (0.11–0.35)	0.59 (0.43–0.80)
Decrease of body temperature after TRY	3.1 (2.1–4.6)	5.4 (3.3–8.7)
Increase of body temperature after TRY	> 10	> 10
Reversal of TRY-induced cyanosis	0.00044 (0.00028–0.00072)	0.00059 (0.00029–0.0012)
Increase of pupil diameter after TRY	> 10	> 10
Decrease of pupil diameter after TRY	> 10	> 10
Stimulation of mortality after TRY	> 10	> 10
Protection from NOR lethality	0.19 (0.11–0.33)	0.26 (0.16–0.41)

[a]Values determined for separate enantiomers were similar

Table 3. Comparison of antiemetic activity in the apomorphine (APO) test in dogs. At different time intervals after subcutaneous (SC) or oral (PO) administration of the test compound, dogs were dosed with APO (0.31 mg/kg, SC). Complete absence of emesis for 1 h after the apomorphine challenge, was adopted as the criterion of antiemetic activity

Antiemetic activity	ED_{50} (mg/kg), mean (95% confidence limits) risperidone	9-hydroxy-risperidone[a]
Time (h) after SC		
1	0.0057 (0.0043–0.0074)	0.0094 (0.0031–0.028)
2	0.0061 (0.0030–0.012)	0.0071 (0.0034–0.015)
4	0.0097 (0.0056–0.017)	0.011 (0.0018–0.072)
8	0.013 (0.0075–0.021)	0.019 (0.0062–0.057)
16	0.014 (0.0080–0.025)	0.020 (0.016–0.026)
32	0.091 (0.053–0.16)	0.10 (0.056–0.18)
Time (h) after PO		
1	0.0071 (0.0044–0.011)	0.011 (0.0066–0.020)
2	0.0079 (0.0041–0.015)	0.010 (0.0078–0.013)
4	0.0079 (0.0047–0.013)	0.0094 (0.0031–0.028)
8	0.011 (0.0081–0.014)	0.011 (0.0066–0.020)
16	0.013 (0.0063–0.026)	0.028 (0.016–0.050)
32	0.095 (0.038–0.24)	0.059 (0.032–0.11)

[a]Values determined for separate enantiomers were similar
Data for risperidone were taken from Janssen et al. (1988)

9-hydroxy-risperidone levels in frontal cortex and striatum were reached at 4 h after administration and amounted to about 0.9 ng-eq./g, which was similar to the corresponding 4-h plasma levels (Fig. 5). Thereafter, concentrations of 9-hydroxy-risperidone declined. Mean residence times of the active metabolite were approximately 12 h in frontal cortex and in striatum, which was nearly 5 times that in plasma. Comparison of AUCs revealed average tissue to plasma ratios of 1.8 for 9-hydroxy-risperidone for frontal cortex and of 1.2 for striatum. The time-course of 9-hydroxy-risperidone in cerebellum was similar to that in plasma, but levels were about ten times lower. Just as for risperidone, concentrations of 9-hydroxy-risperidone in the rest of brain were intermediate between frontal cortex and striatum on the one hand and cerebellum on the other. A comparison of AUCs revealed a tissue to plasma ratio of 0.5 for 9-hydroxy-risperidone in the rest of brain. In all brain regions investigated, tissue to plasma AUC ratios of 9-hydroxy-risperidone were in the order of three times lower than those of risperidone.

In frontal cortex and striatum, risperidone represented most of the NVR till 12 or 8 h after administration, whereas in plasma and in cerebellum, risperidone contributed to less than half of the radioactivity within 2 h after dosing. Comparison of AUCs revealed average risperidone to NVR ratios of 0.7 in frontal cortex, of 0.6 in striatum and 0.2 in cerebellum. In frontal cortex and striatum, the largest fraction of metabolites was due to

9-hydroxy-risperidone. Up to 12 h, other metabolites represented at the utmost 13% of the NVR in frontal cortex and 24% of the NVR in striatum, whereas in cerebellum the metabolites other than 9-hydroxy-risperidone accounted for more than half of the NVR from 4 h after administration.

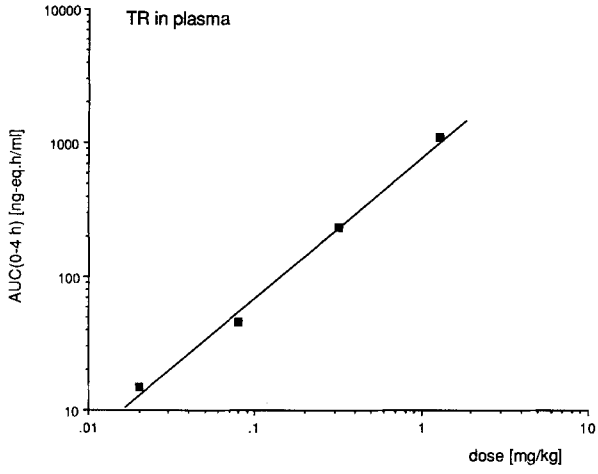

Fig. 2. AUC_{0-4h}-values of total radioactivity (TR) in plasma in the male rat after increasing subcutaneous doses of ^{14}C-risperidone

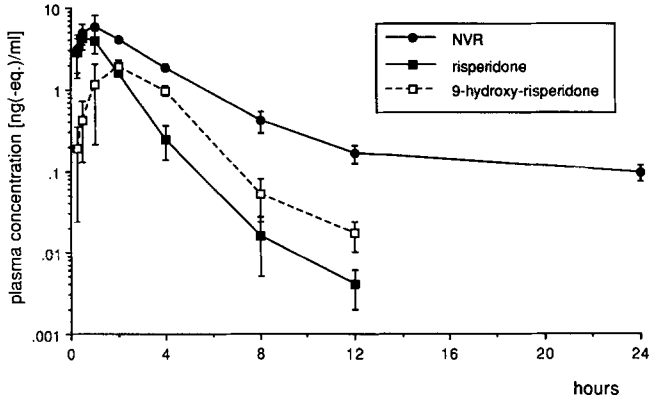

Fig. 3. Mean (± SD, $n = 4$) plasma concentrations of non-volatile radioactivity (NVR), unchanged risperidone and the active metabolite 9-hydroxy-risperidone in the male Wistar rat after single subcutaneous administration of ^{3}H-risperidone at 0.02 mg/kg

In contrast to AUCs of risperidone and 9-hydroxy-risperidone, AUCs of metabolites other than 9-hydroxy-risperidone in frontal cortex and striatum were of the same order as that in cerebellum (Table 4). Besides, frontal cortex to plasma and striatum to plasma concentration ratios of risperidone and of 9-hydroxy-risperidone increased unambiguously during the experiment, whereas corresponding ratios of the sum of metabolites other than 9-hydroxy-risperidone did not (Fig. 6).

Discussion

Plasma concentrations of various agents active in the central nervous system, such as benzodiazepines and neuroleptics, are known to be imperfect predictors of the clinical response (Colburn and Jack 1987; Cohen et al. 1988; Kirch et al. 1988). An inadequate relationship between concentrations of the active moiety in plasma and pharmacological response may be due to a difference between the time-course of the active moiety in plasma and that at the receptor site. Therefore, studies of distribution profiles of active compounds in specific receptor-rich brain regions offer a more direct approach to the examination of pharmacokinetic-pharmacodynamic relationships of neuroleptics. For haloperidol for example, the correlation between serum and brain concentrations in the rat was not strict (Lewi et al. 1970; Öhman et al. 1977). There exists apparently a saturating dose above which the brain concentrations of haloperidol increase very little (Öhman et al. 1977). A good correlation, however, was found between brain levels of haloperidol and its effects on behaviour. Just like risperidone itself, 9-hydroxy-risperidone is a potent antagonist of serotonin, dopamine and noradrenaline. Both compounds are very comparable in potency, pharmacological profile, and onset and duration of action. Therefore, metabolically formed 9-hydroxy-risperidone may contribute to the biological activity of risperidone, but is not expected to result in additional secondary effects.

The ED_{50} of risperidone for central 5-HT_2 antagonism in the separate tryptamine test in rats after subcutaneous administration is 0.02 mg/kg (Janssen et al. 1988; Megens et al. 1992). In the present study, a higher value of 0.13 mg/kg is obtained for the central 5-HT_2 antagonism

Table 4. Mean tissue weights ($n = 32$) of dissected brain regions, and pharmacokinetic parameters of the non-volatile radioactivity (NVR), unchanged risperidone (RIS), the active metabolite 9-hydroxy-risperidone (9-OH) and the sum of other metabolites (OM), as calculated from the difference between NVR and (risperidone + 9-hydroxy-risperidone), in male Wistar rats after single subcutaneous administration of ^{3}H-risperidone at 0.02 mg/kg

Tissue	Weight		Pharmacokinetic parameters (units)												
			T_{max} (h)			C_{max} [ng(-eq.)/ml][a]			$t_{1/2,\beta}$ (h)	MRT (h)		AUC [ng(-eq.)h/ml][a]			
	(mg)	(%)	NVR	RIS	9-OH	NVR	RIS	9-OH	RIS	RIS	9-OH	NVR	RIS	9-OH	OM
Plasma	–	–	1	0.5	2	5.90	4.19	1.90	1.0	1.5	2.5	24.6	8.57	7.14	8.9
Frontal cortex	74.2	4.7	1	1	4	7.55	6.89	0.893	3.9	5.7	11.7	61.4	43.3	12.5	5.6
Striatum	66.5	4.2	1	1	4	6.85	5.84	0.857	2.5	3.8	12.3	42.7	24.3	8.58	9.8
Cerebellum	197	12.6	1	1	2	1.72	1.39	0.183	0.8	1.4	≤ 4	12.0	2.46	0.878	8.7
Rest of brain	1235	78.5	1	1	4	4.35	4.18	0.394	2.6	3.7	7.5	24.6	15.3	3.49	5.8

[a]Concentrations and AUCs of NVR, 9-hydroxy-risperidone and other metabolites were expressed in ng-equivalents (-eq.) of risperidone

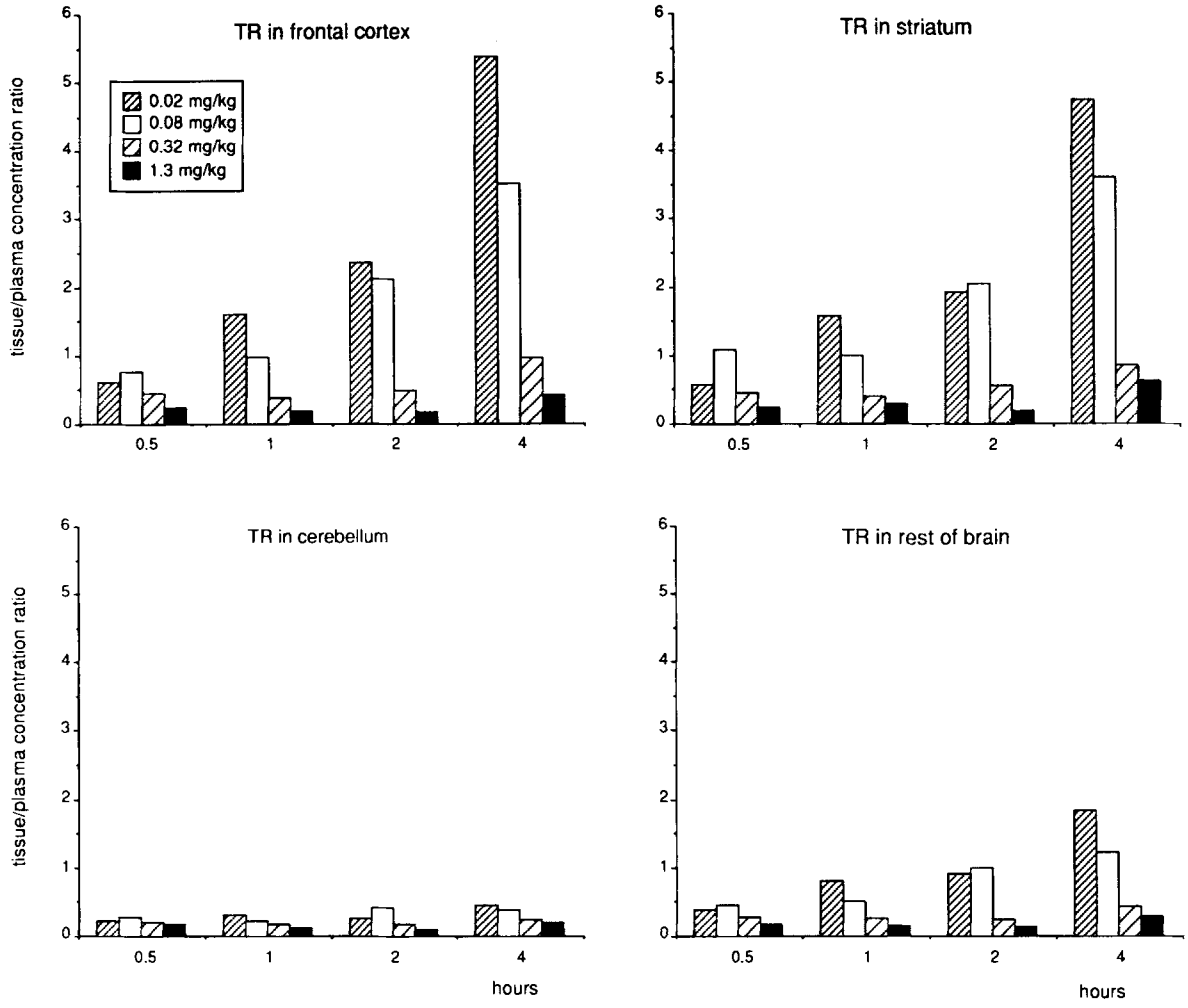

Fig. 4. Mean tissue to plasma concentration ratios of total radioactivity (TR) in frontal cortex, striatum, cerebellum and the rest of brain in the male rat after increasing subcutaneous doses of ^{14}C-risperidone

of risperidone (Table 2) in the successive apomorphine, tryptamine and noradrenaline interaction test (ATN test; Niemegeers et al. 1977). The reason for this apparent discrepancy is twofold: (1) the use of different criteria for the evaluation of the tryptamine antagonism, and (2) in the ATN test, the preceding apomorphine injection may affect the results obtained for tryptamine antagonism (Awouters et al. 1990). After a subcutaneous dose of risperidone at 0.02 mg/kg, in brain regions with high concentrations of 5-HT_2 or D_2 receptors, viz. frontal cortex and striatum, peak times, apparent half-lives and mean residence times of risperidone, as well as of its active metabolite 9-hydroxy-risperidone, were markedly higher than in plasma. By contrast in cerebellum, a brain region with very low concentrations of 5-HT_2 and D_2 receptors, the time-course of both active compounds was similar to that in plasma. Moreover, concentrations of the two active compounds in frontal cortex and striatum were much higher than in cerebellum. The brain regions mentioned are extremes for concentrations of 5-HT_2 and/or D_2 receptors, whereas in the rest of brain average receptor concentrations are intermediate. Concentrations of risperidone and 9-hydroxy-risperidone in the rest of brain were also intermediate between frontal cortex and striatum on the one hand and cerebellum on the other. These observations pointed to the influence of specific receptor binding on the tissue kinetics of the two active compounds. The higher levels of risperidone and 9-hydroxy-risperidone in frontal cortex as compared with striatum correspond with a binding affinity for 5-HT_2 receptors that was even higher than for D_2 receptors. For other metabolites, there was no retention in tissues with high concentrations of 5-HT_2 or D_2 receptors. Apparently, they were not influenced by a binding affinity for these receptors.

Brain to plasma concentration ratios of risperidone were markedly higher than those of 9-hydroxy-risperidone. Therefore, parent risperidone represents a larger fraction of the active moiety at the site of action than in plasma. In rats, the availability of parent risperidone at the site of action was about three times that of metabolically formed 9-hydroxy-risperidone. Hence, after subcutaneous administration in the rat, it is likely that the in vivo receptor occupancy was mainly due to the parent compound.

In plasma and cerebellum, radioactivity concentrations increased dose proportionally between 0.02 and

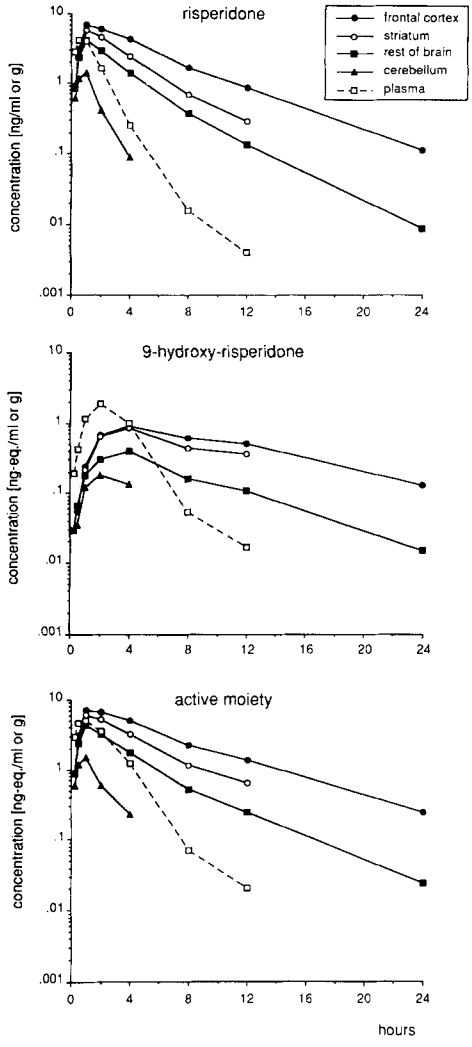

Fig. 5. Mean concentrations of unchanged risperidone, of the active metabolite 9-hydroxy-risperidone and of the active moiety (risperidone + 9-hydroxy-risperidone) in plasma and brain tissues of the male rat after single subcutaneous administration of ^{3}H-risperidone at 0.02 mg/kg

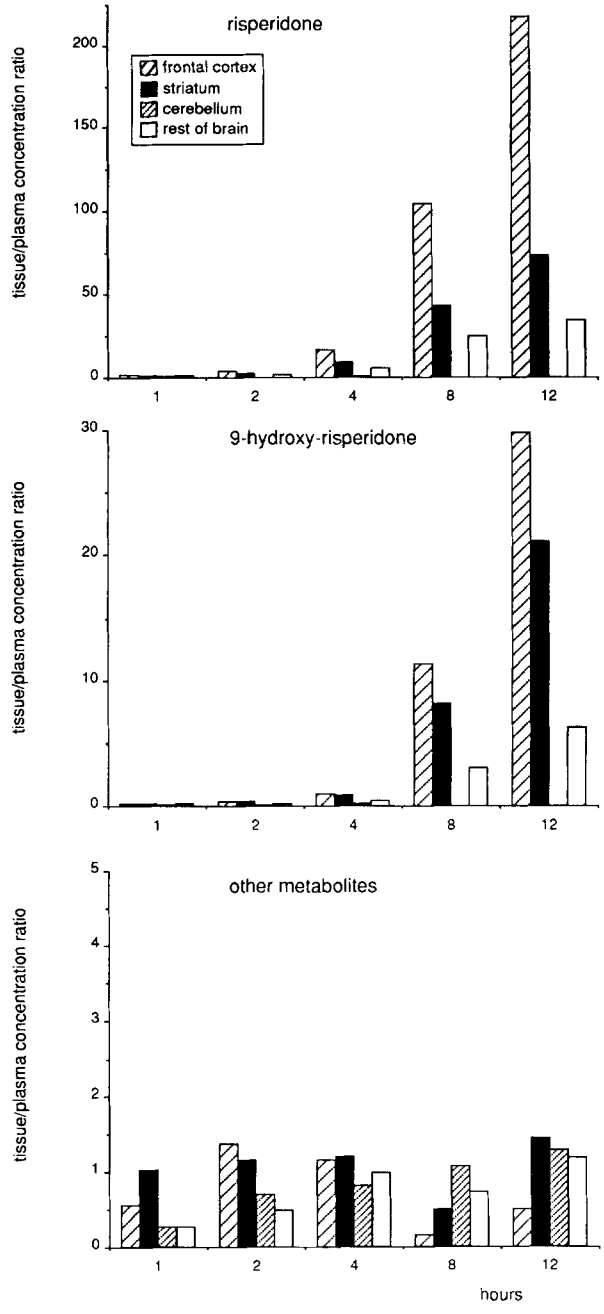

Fig. 6. Mean tissue of plasma concentration ratios of unchanged risperidone, the active metabolite 9-hydroxy-risperidone and of other metabolites in the male rat from 1 h to 12 h after single subcutaneous administration of ^{3}H-risperidone at 0.02 mg/kg

1.3 mg/kg. In frontal cortex and in striatum, however, radioactivity concentrations increased less than dose proportionally. After subcutaneous administration at 0.02 mg/kg, peak times were reached later and half-lives and mean residence times of risperidone, 9-hydroxy-risperidone and of the radioactivity were markedly longer in frontal cortex and striatum than in plasma; however, with increasing dose levels, time courses of radioactivity concentrations in these brain regions became more similar to that in plasma. These results confirm the expectation that the specific receptor binding has less influence on tissue kinetics at higher dose levels, since the fraction of the drugs available in excess at the receptor site will not bind specifically. Within the dose range investigated, probably only the specific receptor binding retards the elimination.

At 0.5 or 1 h after subcutaneous administration of risperidone, ED_{50} values of central 5-HT_2 drug effects in the rat, measured as inhibition of serotonin agonist-induced behavioural effects, were in the order of 0.02 mg/kg (Janssen et al. 1988; Megens et al. 1992). ED_{50} values increased with half-lives of 0.9–1.3 h (calculated from data from Janssen et al. 1988 and Megens et al. 1992) and were 0.1–0.4 mg/kg at 4 h after dosage. After subcutaneous administration of risperidone at 0.02 mg/kg, the elimination rate of risperidone, 9-hydroxy-risperidone and radioactivity in frontal cortex was slower than in plasma and tissue to plasma concentration ratios rapidly increased. After subcutaneous administration of risperidone at higher dose levels, however, tissue to plasma concentration ratios

increased more gradually and elimination half-lives in frontal cortex approached those in plasma. The plasma half-life of the active moiety (risperidone + 9-hydroxy-risperidone) between 2 and 8 h after subcutaneous administration was 1 h. After a subcutaneous dose between 0.1 and 0.4 mg/kg, the half-life of the active moiety in frontal cortex was probably just slightly longer.

Lowest ED_{50} values for central 5-HT_2 antagonistic drug effects were in the order of 0.02 mg/kg and lowest ED_{50} values for central D_2 antagonistic drug effects in the order of 0.1 mg/kg (Janssen et al. 1988; Megens et al. 1992). For both activities, peak effects were observed at 0.5–1 h after subcutaneous administration. Corresponding active concentrations in frontal cortex after administration of 0.02 mg/kg were 3–7 ng/g. If dose proportionality is assumed, corresponding active concentrations in striatum can be extrapolated from the 0.02 mg/kg dose. In this way active striatum levels of 13–30 ng/g can be predicted at 0.5–1 h after subcutaneous administration of risperidone at 0.1 mg/kg. However, since striatum levels, in contrast to plasma levels, increased less than dose proportionally, these values are probably overestimated.

The mean purpose of the present study was to investigate the specific uptake of risperidone and metabolically formed 9-hydroxy-risperidone in different brain regions. In the experiments, the subcutaneous route was chosen in order to evaluate the in vivo pharmacological profile of risperidone in the rat, which was also investigated after subcutaneous administration. Due to the absence of a first-pass metabolism, risperidone and 9-hydroxy-risperidone are available at different concentrations and in a different ratio after subcutaneous administration than after oral administration, which is the present route for therapy. The systemic availability of risperidone and 9-hydroxy-risperidone might differ after any different route of administration. Further, the availability of both compounds is species related, and in rodents it is also sex related. 9-Hydroxy-risperidone to risperidone plasma AUC ratios, for instance, amount to 2.9 after oral administration in male rats, 1.3 after oral administration in female rats and 9.7 after oral administration in dogs (Van Beijsterveldt, Monbaliu, Meuldermans and Heykants, unpublished results). In man, this ratio ranges between extensive and poor metabolizers of debrisoquine from 6 to 0.2 after oral administration and from 3 to 0.1 after either intramuscular or intravenous administration of risperidone (Huang et al. 1993). This already indicates that the subcutaneous route chosen for the present study in male rats, resulting in 9-hydroxy-risperidone to risperidone plasma AUC ratios of 0.8, is not less relevant for the situation in man than the oral or intravenous route. Moreover, although the availability of risperidone and 9-hydroxy-risperidone after subcutaneous administration is different from that after oral administration, the distribution phenomenon described here is clearly relevant. Indeed, the specific uptake of risperidone and 9-hydroxy-risperidone in the different brain regions, as well as their elimination kinetics, will not be different whether risperidone is administered orally or subcutaneously. Tissue to plasma concentration ratios of either risperidone or 9-hydroxy-risperidone will not be different, in so far as there is no interaction between the two active compounds for their distribution to the different brain regions. In order to prove the absence of such an interaction, the regional brain distribution of 9-hydroxy-risperidone in the present study should be compared with that after administration of 9-hydroxy-risperidone itself, i.e. in the absence of risperidone.

In conclusion, after a pharmacologically active dose of risperidone, the pharmacokinetics of both active compounds, risperidone and 9-hydroxy-risperidone, at the site of action were found to be different from that in plasma; peak times were reached later, elimination half-lives and mean residence times were longer and levels increased less than dose dependently. The active metabolite 9-hydroxy-risperidone makes a large contribution to the active moiety in plasma but a smaller contribution to the active moiety at the site of action. This indicates that 9-hydroxy-risperidone contributes to the in vivo activity of risperidone, but to a smaller extent than may be predicted from plasma levels.

These conclusions, obtained after subcutaneous administration of risperidone in rats, are not dependent on the route of administration of risperidone. They would have been exactly the same whether rats would have been dosed orally or by whatever route of administration.

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References

Awouters FHL, Niemegeers CJE, Megens AAHP, Janssen PAJ (1990) Functional interaction between serotonin-S_2 and dopamine-D_2 neurotransmission as revealed by selective antagonism of hyperreactivity to tryptamine and apomorphine. J Pharmacol Exp Ther 254:945–951

Bouthenet M-L, Martres M-P, Salès N, Schwartz J-C (1987) A detailed mapping of dopamine D-2 receptors in rat central nervous system by autoradiography with [^{125}I]iodosulpride. Neuroscience 20:117–155

Chan KK (1982) A simple integrated method for drug and derived metabolite kinetics: an application of the statistical moment theory. Drug Metab Dispos 10:474–479

Claus A, Bollen J, De Cuyper H, Eneman M, Malfroid M, Peuskens J, Heylen S (1992) Risperidone versus haloperidol in the treatment of chronic schizophrenic inpatients: a multicentre double-blind comparative study. Acta Psychiatr Scand 85:295–305

Cohen BM, Babb S, Campbell A, Baldessarini RJ (1988) Persistence of haloperidol in the brain. Arch Gen Psychiatry 45:878–880

Colburn WA, Jack ML (1987) Relationships between CSF drug concentrations, receptor binding characteristics, and pharmacokinetic and pharmacodynamic properties of selected 1,4-substituted benzodiazepines. Clin Pharmacokinet 13:179–190

Gelders YG, Heylen SLA, Vanden Bussche G, Reyntjens AJM, Janssen PAJ (1990) Pilot clinical investigation of risperidone in the treatment of psychotic patients. Pharmacopsychiatry 23:206–211

Gibaldi M, Perrier D (1982) Pharmacokinetics, 2nd edn. Dekker, New York Basel

Huang M-L, Van Peer A, Woestenborghs W, De Coster R, Heykants J, Janssen A, Zylicz Z, Visscher H, Jonkman J (1993) Pharmacokinetics of the novel antispsychotic agent risperidone and the prolactin response in healthy subjects. Clin Pharmacol Ther 54:252–268

Janssen PAJ, Niemegeers CJE, Awouters FHL, Schellekens KHL, Megens AAHP, Meert TF (1988) Pharmacology of risperidone (R 64 766), a new antipsychotic with serotonin-S_2 and dopamine-D_2 antagonistic properties. J Pharmacol Exp Ther 244:685–693

Kirch DG, Bigelow LB, Korpi ER, Wagner RL, Zalcman S, Wyatt RJ (1988) Serum haloperidol concentrations and clinical response in schizophrenia. Schizophrenia 14:283–289

Leysen JE, Geerts R, Gommeren W, Verwimp M, Van Gompel P (1982) Regional distribution of serotonin-2 receptor binding sites in the rat and effects of neuronal lesions. Arch Int Pharmacodyn Ther 256:301–305

Leysen JE, Gommeren W, Eens A, de Chaffoy de Courcelles D, Stoof JC, Janssen PAJ (1988) Biochemical profile of risperidone, a new antipsychotic. J Pharmacol Exp Ther 247:661–670

Leysen JE, Janssen PMF, Gommeren W, Wynants J, Pauwels PJ, Janssen PAJ (1992) In vitro and in vivo receptor binding and effects on monoamine turnover in rat brain regions of the novel antipsychotics risperidone and ocaperidone. Mol Pharmacol 41:494–508

Lewi PJ, Heykants, JJP, Allewijn FTN, Dony JGH, Janssen PAJ (1970) Distribution and metabolism of neuroleptic drugs, part 1: pharmacokinetics of haloperidol. Arzneimittelforschung 20: 943–948

Mannens G, Huang M-L, Meuldermans W, Hendrickx J, Woestenborghs R, Heykants J (1993) Absorption, metabolism and excretion of risperidone in humans. (accepted 1993) Drug Metab Dispos

Megens AAHP, Awouters FHL, Meert TF, Schellekens KHL, Niemegeers CJE, Janssen PAJ (1992) Pharmacological profile of the new potent neuroleptic ocaperidone (R 79598). J Pharmacol Exp Ther 260:146–159

Mesotten F, Suy E, Pietquin M, Burton P, Heylen S, Gelders Y (1989) Therapeutic effect and safety of increasing doses of risperidone (R 64 766) in psychotic patients. Psychopharmacology 99:445–449

Meuldermans W, Hendrickx J, Mannens G, Lavijsen K, Janssen C, Bracke J, le Jeune L, Lauwers W, Heykants J (accepted 1993) The metabolism and excretion of risperidone after oral administration in rats and dogs. Drug Metab Dispos

Niemegeers CJE (1982) Antiemetic specificity of dopamine antagonists. Psychopharmacology 78:210–213

Niemegeers CJE, Lenaerts FM, Artois KSK, Janssen PAJ (1977) Interaction of drugs with apomorphine, tryptamine and norepinephrine. A new "in vivo" approach: the ATN test in rats. Arch Int Pharmcodyn Ther 227:238–253

Öhman R, Larsson M, Nilsson IM, Engel J, Carlsson A (1977) Neurometabolic and behavioural effects of haloperidol in relation to drug levels in serum and brain. Naunyn-Schmiedeberg's Arch Pharmacol 299:105–114

Pazos A, Cortés R, Palacios JM (1985) Quantitative autoradiographic mapping of serotonin receptors in the rat brain. II. Serotonin-2 receptors. Brain Res 346:231–249

Sunderland T, Cohen BM (1987) Blood to brain distribution of neuroleptics. Psychiatry Res 20:299–305