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Short communication

THE REGIONAL LOCALISATION OF A NEW POTENT CENTRALLY ACTING ANTIHYPERTENSIVE AGENT R 28935 AND ITS LESS ACTIVE THREO-ISOMER R 29814 IN THE CAT BRAIN

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Systemic administration of the centrally acting antihypertensive agent R 28935 to cats resulted in a long lasting decrease of mean arterial pressure (±30%) whereas the same dose of the threo-isomer R 29814 was ineffective. The antihypertensive activity was due to the unaltered drug. In spite of an identical log P, pKa, dose and a comparable plasma level, the concentration of R 28935 in all the brain areas tested was about twice that of the threo-isomer, suggesting a stereoselective uptake and/or binding of R 28935.

Regional brain distribution Antihypertensive agents Centrally acting Cats
Stereoselective uptake

1. Introduction

R 28935 (erythro-1-{1-[2-(2,3 dihydro-1,4 benzodioxin-2-yl)-2-hydroxyethyl]-4-piperidinyl}-1,3-dihydro-2H-benzimidazol-2-one) (fig. 1) developed in a structure—activity relationship program for antihypertensive agents by Van Wijngaarden and Soudijn (1975), is a potent long-acting centrally active antihypertensive drug whose mechanism of action is unknown (Van Zwieten, 1975; Wellens et al., 1975a,b).

Its threo-isomer is at least ten times less active. The lipophilicity and the dissociation constant are the same for both diastereoisomers (log P = 3.43, pKa = 7.64); the difference in potency can thus not be explained by a difference in distribution in the central nervous system due to these physicochemical parameters but may result from a difference in the rate and route of elimination of the diastereoisomers and/or from a stereoselective uptake and/or binding mechanism.

In the present study the regional distribution of the tritium-labelled isomers in the cat brain was estimated after systemic administration of a 30 μg per kg dose which resulted in a substantial decrease of blood pressure in the R 28935-treated cats only.

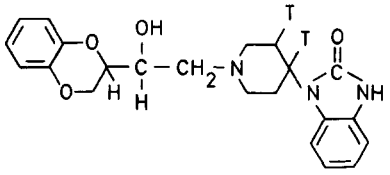

Fig. 1. Structure of R 28935 (1-(erythro-1-{1-[2-(2,3 dihydro-1,4 benzodioxin-2-yl)-2-hydroxyethyl]-4-piperidinyl-3,4-t_2}-1,3-dihydro-2H-benzimidazol-2 one) and position of the tritium label.

2. Materials and methods

2.1. Chemistry

Specially tritium-labelled R 28935 and R 29814 were synthesized on a millimolar scale (Van Wijngaarden et al., 1977) with a specific activity of 9 Ci/mM. The chemical structure and the position of the label are shown in fig. 1.

2.2. Animal studies

After an habituation period of 3 days, male adult cats weighing 2.5—4.0 kg, were anaesthetized with α-chloralose (60 mg/kg i.p.). After endotracheal intubation the animals were artificially ventilated and heparinized (about 1000 IU/kg i.v.). Blood pressure was recorded continuously from the femoral artery via a Statham P32 pressure transducer connected to a Hellige recorder. The only cats used were those which, after an equilibrium period of 30 min, showed a mean arterial pressure of over 100 mm Hg. Freshly prepared saline solutions of the labeled compounds, diluted with the unlabeled reference compounds to a specific activity of 0.9 Ci/mM were slowly injected into the left femoral vein at a dose of 30 μg/kg (0.1 ml/kg). 30 min later the cats were killed by the injection of air, after 20 ml of femoral arterial blood had been collected. The brain was immediately removed, washed with saline, cooled on ice and dissected in the following sequence; pituitary gland, cerebellum myelencephalon, pons, hypothalamus, rest of the brain. The brain parts were stored at −20°C.

2.3. Sample preparation

The thawed and weighed brain parts were extracted with 70% isopropyl alcohol by repeated (3 ×) homogenization (Ultra Turrax desintegrator 20,000 rpm) and centrifugation (3500 rpm). Extraction of the radioactive material by this method was complete. An aliquot of the combined supernatants was assayed for radioactivity using Insta-Gel (Packard) as the scintillator solution. The radioactivity of the blood was estimated by combusting 0.2 ml aliquots in a Packard Tri-Carb sample oxidizer and determining the radioactivity of the subsequently formed water in Insta-Gel. Plasma was assayed directly in Insta-Gel. Counting efficiency was determined by internal standardization.

2.4. Assay of R 28935 and R 29814 by the inverse isotope dilution method

2.4.1. R 28935

An accurately weighed amount (100 mg) of R 28935 was dissolved in 10 ml of methanol acidified with an excess in cyclohexylsulfamic acid. An aliquot of plasma (1 ml) or of brain extract (5 ml) was added and thoroughly mixed. The amount of radioactivity in the aliquots must be known and sufficiently large. After evaporation to dryness of the mixture under a current of nitrogen in a water bath at 50°C, R 28935 cyclohexylsulfamate was isolated and purified by several recrystallizations from different solvents (70% isopropyl-alcohol, 80% ethyl alcohol) until the specific activity of the crystals in dpm per mg remained constant. The R 28935 content was calculated from x = 100 sm/b in which s = specific activity of the purified drug in dpm per mg, m = weight of the added R 28935 in mg, b = the radioactivity of the added aliquot in dpm (Soudijn and Van Wijngaarden, 1972).

2.4.2. R 29814

The pure threo-isomer separated in low yield by counter current distribution from a mixture of the diastereo isomers was only available in a limited amount. By combining preparative thin layer chromatography and the inverse isotope dilution technique as described for R 28935, it was possible to use only 30 mg of R 29814 per assay. After mixing and evaporation as described for R 28935 the residue was extracted with chloroform after alkalinization with concentrated ammonia. The chloroform extract was evaporated down

TABLE 1

Concentration of R 28935 and of its threo-isomer R 29814 in blood, plasma and different areas of the cat brain.

	R 28935 (erythro-isomer)			
Cats	A	B	C	
Weight in kg	4.1	4.1	3.4	
% decrease of MAP	35.9	29.7	26.0	
Tissue	pg R 28935/mg of wet tissue			Mean ± S.E.M.
Blood [1]	8.0	8.6	9.7	8.8 ± 0.52
Plasma [1]	9.8	9.1	12.3	10.4 ± 0.97
Plasma	7.0	5.5	6.1	4.2 < 6.2 ± 0.44
Pituitary gland	147.1	108.5	104.6	120.1 ± 13.56
Myelencephalon	35.5	31.8	27.7	31.7 ± 2.25
Hypothalamus	36.9	34.3	31.9	34.4 ± 1.44
Pons	42.1	36.0	31.6	36.6 ± 3.04
Cerebellum	43.8	37.1	32.0	37.5 ± 3.33
Rest of the brain	41.8	37.5	33.4	37.6 ± 2.43
% of the dose present in the brain	0.97	0.79	0.88	0.88 ± 0.005

	R 29814 (threo-isomer)				Difference
Cats	D	E	F		R 28935 vs. R 29814 *t*-test *p*
Weight in kg	3.3	3.0	2.6		
% decrease of MAP	ns [2]	ns	ns		
Tissue	pg R 29814/mg of wet tissue			Mean ± S.E.M.	
Blood [1]	19.0	16.5	14.4	16.6 ± 1.33	0.006
Plasma [1]	31.4	28.2	20.8	26.8 ± 3.14	0.009
Plasma	11.4	10.0	6.8	17.4 < 9.4 ± 1.36	0.089
Pituitary gland	89.8	72.7	108.8	90.4 ± 10.43	0.158 ns
Myelencephalon	13.2	11.8	14.6	13.2 ± 0.81	0.003
Hypothalamus	17.7	12.9	17.2	15.9 ± 1.52	0.002
Pons	14.2	13.3	16.1	14.5 ± 0.83	0.004
Cerebellum	15.4	13.0	16.9	15.1 ± 1.14	0.004
Rest of the brain	15.1	13.1	15.8	14.7 ± 0.81	0.002
% of the dose present in the brain	0.47	0.37	0.56	0.47 ± 0.055	0.007

[1] Total radioactivity levels expressed as pg unaltered substance per µl.
[2] Not significant.

to a small volume and applied to 20 × 20 cm preparative silica gel thin layer plates (Merck Kieselgel 60 F_{254} 0.25 mm layer thickness). The plate was developed with a mixture of 1% diethylamine in methanol, by allowing the solvent to run a distance of 17 cm. After location of the R 29814 zone using 254 nm U.V., the zone was scraped off and eluted

with methanol. After filtration and evaporation of the filtrate under a current of nitrogen, the residue was dissolved in 1—2 ml of ethyl alcohol, filtrated through Millipore filter (HAWP 02500, 0.45 μ) and concentrated down to a small volume under nitrogen. Then 80% ethyl alcohol and cyclohexylsulfamic acid were added. The mixture was heated and R 29814 cyclohexylsulfamate crystallized on cooling. After two recrystallizations from 80% ethyl alcolhol the specific activity in dpm per mg of the crystals remained constant.

3. Results

The results are summarized in table 1. Systemic injection of R 28935 in a dose of 30 μg per kg resulted in a long lasting lowering of the MAP by about 30% of the initial value in all cats, whereas the blood pressure of animals injected with the same dose of the threo-isomer did not decrease significantly. The radioactivity level in plasma of cats injected with the threo-isomer was 2.6 times the level found in cats treated with R 28935, however the concentrations of both unaltered threo-isomer and R 28935 differed much less, the threo level being 1.5 times the R 28935 level with a statistically low significance of the differences due to the small number of experiments. The amount of radioactivity of whole blood was less than that of plasma in all experiments, indicating that the unaltered substances and their metabolites were not extensively bound to the formed elements of the blood. From the data in table 1 and an assumed haematocrit of 45% for cat blood, it can be estimated that of the 8.8 pg/μl of R 28935 + its metabolites, 3.1 pg (35%) was bound to blood cells and only 1.9 pg (11%) of the 16.6 pg/μl of its threo-isomer + metabolites. In the brain areas tested however, the concentration of R 28935 was about twice that of the threo-isomer and no regional difference in the drug level was found. Inverse isotope dilution showed that after R 28935 administration all the radioactivity in the brain areas was due to unaltered R 28935. After treatment with R 29814, 83% of the radioactivity was due to unaltered threo-isomer and 17% to its metabolites, possibly a reflection of the high level of metabolites in the plasma. Half an hour after the injection, about 0.88% of the dose of R 28935 and about 0.47% of the threo-isomer was present in the brain.

4. Discussion

In spite of an identical logP, pKa and a comparable plasma level of the diastereoisomers, the ratio of brain concentration to plasma level was 6.1 for the antihypertensive agent R 28935 and 1.6 for its much less active threo-isomer. The high level of threo metabolites in plasma may be an indication that the threo-isomer is metabolically less stable than the erythro isomer, although poor penetration and/or binding of the metabolites of the threo-isomer to the tissues may also be an explanation. It is improbable that the metabolites of the threo-isomer interfered with the uptake of intact R 29814, as preliminary experiments showed that the same difference in brain concentration existed after infusion of both diastereoisomers via the vertebral artery. With this technique the substances are administered directly to the brain, thus preventing possible interference of metabolites with the uptake of the isomers. The plasma level of the intact threo-isomer tends to be higher than that of the erythro form, and while higher plasma protein binding could prevent penetration of the threo-isomer into the brain such strong binding would also protect the compound from extensive metabolic degradation.

Thus the difference in brain concentration and in potency of the two diastereoisomeric antihypertensive agents is much more likely to be due to a stereoselective uptake and/or binding mechanism for R 28935. This stereoselective binding might operate in other

tissues as well. The differences in binding of the diastereoisomers and its metabolites to the blood cells makes this plausible. No regional differences in the concentration of the diastereoisomers could be demonstrated and accumulation in one of the brain areas could not serve to indicate a site of action. We are of course aware that a higher concentration in a distinct brain area is not necessarily an indication for the site of action. On the other hand the finding that a pharmacologically active substance accumulated in a brain area must not be ignored and merits further investigation, as specific binding to receptor sites can be masked by a binding to sites of loss. Based on the fact that all of the radioactivity in the brain areas originated from unaltered R 28935 and that the blood pressure lowering effect had a very rapid onset, it is likely that the hypotensive activity was due to the compound itself and not to its metabolites.

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