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The Regional Localization of R28935 in the Cat Brain as Dependent on the Route of Administration*

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Summary. Intravenous injection of the antihypertensive agent R28935 and its pharmacologically less active threo-isomer R29814 resulted in a distribution profile in the cat brain which differed from the regional localization after administration via the left vertebral artery. Although the two isomers had the same physicochemical properties, R28935 penetrated more readily into the CNS.

Intravenous administration resulted in almost equal levels in all brain parts, whereas after injection into the vertebral artery caudal structures contained more of both compounds than rostral structures. Differences existed between the concentrations in homotopic brain areas, especially in the brain stem.

From comparison of the levels of R28935 after injection of an equiactive dose either i.v. or into the vertebral artery it is tempting to speculate that the mesencephalic tegmentum, the nucleus of the solitary tract, the inferior colliculi and/or the locus coeruleus are possible sites of the hypotensive action.

Key words: R28935 – Antihypertensive agents – Cat brain – Regional brain distribution.

Introduction

Low doses of R28935 (*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), an antihypertensive analogue of pimozide developed by Van Wijngaarden et al. (1975), induce a long lasting and considerable reduction in arterial blood pressure and heart rate of cats, spontaneous hypertensive rats and dogs. A central mechanism of action, not related to stimulation of central α-adrenoceptors, has been postulated (Finch, 1975; Wellens et al., 1975a, 1975b, 1975c; Van Zwieten, 1975) and was reflected by the decrease in sympathetic nerve activity induced by the compound (Taylor and Antonaccio, 1978). The antihypertensive activity is due to the unaltered drug (Loonen et al., 1977).

The threo-isomer of R28935, named R29814, is far less active but has the same pharmacological properties (Van Zwieten, 1975). We have already demonstrated that although both isomers have the same log P (3.43; octanol-Sörensen buffer pH = 9.8) and pKa (7.64; titration in methanol water mixtures) (Dr. J. Peeters, Janssen Pharmaceutica, personal communication), intravenous injection of the same doses results in a brain concentration of R28935 which is about twice that of the threo-isomer (Loonen et al., 1977). In the present paper the regional distribution after injection of both isomers into the left vertebral artery at a dose of 3 μg/kg is described. From a comparative study of the regional concentration of R28935 after i.v. administration or injection into the vertebral artery of equiactive doses, it may be possible to identify putative sites of action. Only structures showing identical concentrations of R28935 after intravenous or vertebral artery administration may be considered as putative sites of action.

Materials and Methods

Drugs. Tritium labelled R28935 and R29814 were synthetised with a specific activity of 9 Ci per millimole (Van Wijngaarden et al., 1978). In Fig. 1 the chemical structure and the position of the radioactive label are shown.

Animals. Male cats (2.5–5.0 kg) were anaesthetized with α-glucochloralose (60 mg/kg i.p.). The animals were intubated and positively ventilated. The left femoral vein was cannulated and about

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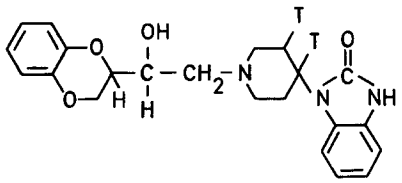

Fig. 1. Structural formula of R28935: erythro-1{1-[2-(2,3-dihydro-1,4-benzodioxin-2-yl)-2-hydroxyethyl]-4-piperidinyl}-1,3-dihydro-2H-benzimidazol-2-one and the position of the radioactive label

3000 I. U. of heparin were injected. Blood pressure was recorded continuously from the femoral artery via a Statham P 23 Db pressure transducer on an Hellige HE-17 recorder. The first three ribs were cut near the sternum. With exception of the vertebral artery, all side branches of the left subclavian artery were ligated and subsequently a cannula was introduced in the latter artery in the proximal direction. Because the tip of the cannula was brought into a position just distal to the bifurcation of the subclavian and vertebral arteries, injected solutions were carried to the CNS by the bloodstream. Detailed descriptions of this technique are given in the literature (Henning and Van Zwieten, 1968; Sattler and Van Zwieten, 1967; Wellens et al. 1975d). I.v. injections were made into the femoral vein. All cats had a mean arterial pressure (vide infra) of more than 100 mm Hg after an equilibrium period of thirty min. Freshly prepared saline solutions of the labelled compounds were slowly infused. The dose was 3 μg/kg when administered via the vertebral artery and 30 μg/kg when given i.v. The administered volume was 0.1 ml/kg. After 30 min 20 ml of femoral arterial blood was collected and the cats were killed by injection of air. The brain was extirpated, washed with saline to remove traces of blood, cooled on ice and dissected. The different parts of the brain were stored at −20° C. Homotopic brain areas were assayed separately. After injection of the erythro-isomer those cats were selected for analysis, which showed a fall in blood pressure of about 30%.

Sample Preparation. The total radioactivity in whole blood was determined by combustion of 0.2 ml aliquots in a Packard Tri-Carb Sample Oxidizer and counting the radioactivity in Insta-Gel® (Packard) using a Packard Tri-Carb Liquid Scintillation Spectrometer model 3255. Plasma was prepared by centrifugation of heparinized blood samples (2000 g, 30 min) and the radioactivity was measured after mixing 1 ml of plasma with 10 ml of Insta-Gel®.

The compounds were extracted from the different parts of the brain with 70% isopropylalcohol by means of repeated (3×) homogenization in a Ultra Turrax desintegrator (20,000 rpm) followed by centrifugation. All supernatants were combined and assayed for radioactivity. There remained no radioactivity in the extracted pellets. The amount of unchanged R28935 was assayed by the inverse isotope dilution method as described by Loonen et al. (1977). Counting efficiency was determined by internal standardization.

Calculations. All values represent mean ± standard error of the mean (SEM). The blood pressure lowering is expressed as percentage of the initial mean arterial pressure (MAP), calculated according to the formula: $MAP = \frac{2 \times P_d + 1 \times P_s}{3}$ in which P_d stands for the diastolic and P_s for the systolic blood pressure. Unless stated otherwise the concentration of the compounds in plasma and in the different brain areas is expressed as pg unchanged compound per microliter or milligram wet weight, calculated from the total radioactivity. The concentration in the brain as a whole (Table 2) has been determined by summation of the radioactivity present in each individual brain part and subsequent division by the sum of their weights.

The results were subjected to the one-tailed Student's *t*-test (NEN 1047, p 5.3; Dutch Normalization Institute, Rijswijk) and significant differences were accepted for values of p smaller than 0.05.

Results

At the moment of sacrifice, i.e. 30 min after administration of 3 μg R28935/kg into the vertebral artery, the fall in MAP was 28.4 ± 3.0% ($n = 3$). I.v. injection of 30 μg/kg resulted in a decrease of 28.3 ± 4.7% ($n = 3$), a similar value. This effect was still about 2/3 of the maximum (Van Zwieten, 1975). Injection of the same doses of the threo-isomer did not induce a significant change of the MAP ($n = 3$ for both administration routes). The percentage of the dose which was present in the brain amounted to 5.36 ± 0.98% and 1.08 ± 0.15% after i.a. and i.v. injection of R28935 respectively, whereas the corresponding values for the threo-isomer were 2.80 ± 0.60% and 0.41 ± 0.02%. Inverse isotope dilution showed that after i.a. administration of R28935 99.4 ± 1.9% of the radioactivity in all brain areas was due to the unaltered compound, whereas i.v. injection resulted in a value of 92.5 ± 3.0%, which is not significantly different. The plasma concentrations after i.v. injection were 13.3 ± 2.3 ng R28935/ml and 33.9 ± 8.2 ng R29814/ml, as compared to 2.2 ± 0.2 ng R28935/ml and 1.5 ± 0.2 ng R29814/ml after i.a. administration.

R28935 and R29814 were both evenly distributed over the different brain areas after injection into the left femoral vein. The pituitary gland accumulated both compounds more efficiently than the other parts of the brain (Table 1). After i.a. administration the compounds were no longer evenly distributed. The concentrations in the caudal brain structures were much higher than in the more rostral parts. Moreover the vestibular nucleus, the nucleus of the solitary tract and other areas in the lower brainstem displayed large differences in the drug content of homotopic brain parts. There was also a difference between the distribution profile of the erythro- and the threo-isomer. This was indicated by the ratio between the concentration in a specific area and the concentration in the brain as a whole (Table 2). This ratio tended to be somewhat lower in the caudal part of the medulla oblongata for R29814 than it was for the erythro-isomer. As shown in Table 2 the presented ratio declines rapidly in more rostrally situated structures. In the case of R29814 the decrease is less pronounced than with R28935. It indicates that R29814 penetrates somewhat slower into the brain than R28935.

In order to identify a possible site of action we corrected for the differences in the concentration of R28935 reached in two corresponding contralateral brain areas. Therefore the logarithmic value of the

Table 1. Concentration of R28935 and of its threo-isomer R29814 in different areas of the cat brain. Concentrations are calculated from total radioactivity and expressed as pg unaltered substance per mg wet weight. The semicolon seperates the levels in homotopic brain areas. Each value represents the mean of 3 experiments. For abbreviations, see Table 2

	R28935 (erythro-isomer)				R29814 (threo-isomer)			
	intra-arterial administration (left vertebral artery)		intravenous administration (left femoral vein)		intra-arterial administration (left vertebral artery)		intravenous administration (left femoral vein)	
	left	right	left	right	left	right	left	rigth
Pituitary gland	14.7		73.2		9.2		71.0	
Spinal cord	44.6		19.8		12.8		6.5	
Area postrema	96.7		27.0		23.4		12.0	
NTS	312.1	8.2	30.0	32.4	78.4	1.4	9.5	9.4
Vestibular nucleus (med. sup)	255.1	35.5	28.8	32.4	59.2	9.3	11.9	13.4
Locus coeruleus (p.c.m.)	95.6	48.7	29.4	30.1	43.4	40.0	10.4	10.9
Area ovalis	187.8	20.0	25.9	25.8	59.6	6.3	9.4	9.2
Pons	109.6	69.0	26.8	27.0	70.4	37.4	10.2	10.8
Metencephalic tegmentum	83.7		29.3		61.9		10.5	
Mesencephalic tegmentum	37.7	32.8	30.8	34.1	39.3	27.3	9.0	10.1
Lamina tecti (caudal)	26.1	31.2	32.4	33.0	30.4	15.5	12.7	12.5
Lamina tecti (rostral)	11.6	12.1	33.9	32.7	19.3	9.8	12.5	12.4
Hypothalamus (posterior part)	24.9	25.2	30.2	32.0	35.4	17.6	12.5	12.1
Hypothalamus (anterior part)	5.9	6.2	30.5	29.1	2.0	1.7	11.4	10.7
Thalamus	12.6	6.4	31.3	27.2	12.5	6.7	14.8	15.9
Caudate nucleus	6.6	6.2	36.3	37.0	1.5	1.6	14.1	15.0
Hippocampus	9.1	6.5	34.6	36.4	9.3	4.1	13.1	13.0
Amygdala	7.4	7.1	36.4	35.2	1.2	1.5	13.0	13.2
Olfactory tubercle	5.1	5.6	31.7	26.2	1.5	1.3	12.3	12.3
Mesocortex	7.8	6.9	35.5	34.4	2.0	1.8	13.8	14.2
Middle ectosylvian gyrus	6.7	7.2	39.1	41.2	1.8	1.6	14.7	17.0

Table 2. Ratio between the concentration in some brain areas and the concentration in the brain as a whole. Each value represents mean ± S.E.M. NTS = nucleus of the solitary tract (part ventral to the area postrema). Vestibular nucleus (med. sup.) = medial and superior part of the vestibular nucleus. AFFV = Anterior part of the floor of the fourth ventricle. Locus coeruleus (p.c.m.) = medial compact part of the locus coeruleus

	R28935 (erythro-isomer) 3 µg/kg intra-arterial administration (left vertebral artery)		R 29814 (threo-isomer) 3 µg/kg intra-arterial administration (left vertebral artery)	
	left	right	left	right
NTS	15.0 ± 1.43	0.4 ± 0.10	8.8 ± 3.04	0.2 ± 0.03
Vestibular nucleus (med. sup.)	11.9 ± 0.76	1.6 ± 0.27	6.8 ± 0.98	1.1 ± 0.27
Area ovalis	9.3 ± 0.93	1.0 ± 0.18	6.8 ± 1.13	0.7 ± 0.27
AFFV	4.6 ± 0.64	3.1 ± 1.31	4.7 ± 1.08	3.7 ± 1.09
Locus coeruleus (p.c.m.)	4.4 ± 0.35	1.9 ± 0.74	4.8 ± 0.91	4.5 ± 1.14
Pons	5.0 ± 0.29	2.7 ± 0.92	8.0 ± 0.71	4.3 ± 0.66
Mesencephalic tegmentum	1.7 ± 0.34	1.3 ± 0.57	4.3 ± 0.94	3.0 ± 0.59
Tectum (caudal)	1.3 ± 0.42	1.2 ± 0.66	3.4 ± 0.67	1.7 ± 0.41
Tectum (rostral)	0.6 ± 0.17	0.5 ± 0.14	2.1 ± 0.57	1.1 ± 0.39

actual concentration (expressed as cpm/mg wet weight) in each brain part was calculated. Subsequently the values of homotopic brain areas were averaged. Table 3 shows the results. In most of the structures significant differences existed between the values after i.v. and i.a. injection ($P<0.05$). These areas could therefore be excluded as possible sites of the hypotensive action. For the remaining areas the alternative hypothesis had to be accepted indicating that the concentration of the drug was independent of the applied route of administration.

Table 3. Comparison of the average of the logarithms of the concentrations of R28935 in homotopic brain areas after two routes of administration. Each value represents mean ± S.E.M. for three cats. The concentrations are exposed in cpm per mg wet weight (1 pg = 4.5 cpm). SD = significantly different

	Log concentration ± S.E.M.		Difference	Significance
	intra-arterial administration (left vertebral artery) 3 μg/kg (n = 3)	intravenous administration (left femoral vein 30 μg/kg (n = 3)	intra-arterial vs. intravenous t-value	P
Pituitary gland	1.7997 ± 0.0815	2.5106 ± 0.0406	7.808	0.0013 SD
Olfactory bulb	1.3489 ± 0.1049	2.0285 ± 0.0742	5.289	0.0038 SD
Olfactory tubercle	1.3654 ± 0.0769	2.0974 ± 0.0691	7.080	0.0017 SD
Cingulate gyrus	1.4471 ± 0.0832	2.1541 ± 0.0600	6.892	0.0018 SD
Middle ectosylvian gyrus	1.4733 ± 0.0883	2.2348 ± 0.0921	5.968	0.0027 SD
Mesocortex	1.5009 ± 0.0826	2.1860 ± 0.0536	6.958	0.0018 SD
Corpus callosum	1.2338 ± 0.1528	1.9950 ± 0.1126	4.010	0.0080 SD
Amygdala	1.4682 ± 0.1388	2.1901 ± 0.0704	4.639	0.0049 SD
Hippocampus	1.4863 ± 0.1415	2.1864 ± 0.0764	4.354	0.0061 SD
Caudate nucleus	1.4298 ± 0.1096	2.1982 ± 0.0838	5.570	0.0032 SD
Thalamus	1.5406 ± 0.1482	2.1120 ± 0.0257	3.799	0.0096 SD
Hypothalamus (anterior part)	1.3975 ± 0.1223	2.1125 ± 0.0721	5.036	0.0037 SD
Hypothalamus (posterior part)	1.7333 ± 0.3384	2.1250 ± 0.0889	1.414[a]	0.13
Tectum (rostral)	1.6319 ± 0.1468	2.1580 ± 0.0811	3.137	0.017 SD
Tectum (caudal)	1.9415 ± 0.1914	2.1441 ± 0.0968	0.945	0.20
Mesencephalic tegmentum	2.0532 ± 0.2533	2.1488 ± 0.0678	0.365	0.37
Metencephalic tegmentum	2.4958 ± 0.2010	2.1011 ± 0.0835	1.813	0.072
Pons	2.4697 ± 0.2504	2.0592 ± 0.0973	1.528	0.10
Locus coeruleus (p.c.m.)	2.3542 ± 0.2541	2.0943 ± 0.0708	0.985	0.19
AFFV	2.4355 ± 0.3152	2.0825 ± 0.0861	1.080	0.17
Vestibular nucleus (med. sup.)	2.5668 ± 0.1760	2.0955 ± 0.0767	2.455	0.035 SD
NTS	2.2819 ± 0.1743	2.1660 ± 0.0235	0.659	0.27
Area ovalis	2.4191 ± 0.0882	2.0486 ± 0.0762	3.179	0.017 SD
Spinal cord	2.4485 ± 0.0394	1.9381 ± 0.0546	6.684*	0.0029 SD

* Degree of freedom = 3

This is in most probability true for the mesencephalic tegmentum, the nucleus of the solitary tract followed by the caudal tectum and the locus coeruleus (pars compacta medialis).

Discussion

In a recent study (Loonen et al., 1977) we have demonstrated that the antihypertensive agent R28935 penetrates more readily into the CNS than its less active threo-isomer (R29814). We have suggested the existence of a stereospecific uptake and/or binding mechanism. This hypothesis is further supported by the present results. After i.v. infusion or infusion into the vertebral artery the percentage of the total dose in the brain is higher for the erythro-isomer than it is for the threo-isomer. After injection into the vertebral artery of cats the larger part of the administered compound passes through the vascular bed of the brain before being distributed over the rest of the body (Wellens et al., 1975d). Thus the bulk of the drug is presented directly to the brain. The course of the fall in blood pressure with a very large response during the first few minutes followed by a rapid decrease to a certain plateau value (data not shown), indicates that initially a high brain concentration is achieved, with subsequent decline to a lower steady state value. I.v. administration probably results in a more gradual diffusion of the compounds into the central nervous system. The persistence of differences in the penetration capacity of the diastereoisomers, in spite of different routes of administration, may be used as an argument in favour of specificity of the phenomenon. Neither strong plasma protein binding of the threo-isomer nor interference of metabolites with the uptake of intact R29814 seems to account for the described phenomena (Loonen et al., 1977).

I.v. injection does not result in regional differences in the brain concentrations of R28935. Only in the pituitary gland the concentrations were higher than in the rest of the brain, a fact that may be attributed to the absence of a blood brain barrier and to the extensive vascularization of this organ. The similarity of the levels of R28935 and R29814 in the hypophysis suggest,

that the high concentrations reached here, reflect accumulation due to (aspecific) physicochemical processes. The distribution profile after i.a. administration reflects the relative distribution of vertebral arterial blood (Wellens et al., 1975d). Some differences were observed between the distribution profile of R28935 and R29814. This can be explained by a less active uptake mechanism for the latter drug in combination with the specific pharmacokinetic characteristics of this route of administration. When the amount which is taken up in the proximal part of the vertebral vascular bed is small, more drug remains in the blood and is presented to more distally situated tissue. Therefore the described difference in brain levels and profile may be due to a poorer penetration of R29814 into the brain tissue. However this does not exclude the presence of a stereoselective retention mechanism for R28935.

In order to identify a possible site of action of R28935, we have compared the brain concentrations after administration via two different routes. As in both groups the hypotensive effect is similar, the drug concentration at the site of action should also be of similar magnitude. This effective concentrations is probably adequately reflected by the total tissue level 30 min after injection when the hypotensive effect was stable. We assume that left and right brain areas are of equal importance for the final effect. Therefore we have calculated the average of the concentrations in homotopic structures. The logarithmic values are used as these are – according to the "drug-receptor theory" and to vast experimental information – over a wide range proportional to the biological effect. As a result of these calculations several brain areas can be excluded as sites of action. Of the remaining structures especially the mesencephalic tegmentum, the nucleus of the solitary tract (in contrast to surrounding areas), the inferior colliculi and the locus coeruleus are brain areas in which the level of the antihypertensive drug R28935 is independent of the way of administration, i.v. or into the vertebral artery. We therefore postulate that the hypotensive action of R28935 may be achieved by interaction within one of these brain structures in the indicated order of probability. In order to draw more definite conclusions the experiments should be complemented with measurements at different dose levels and with alternative routes of administration, e.g. intracerebroventricular injection. The present procedure may be a new approach to disclose sites of action for centrally acting hypotensive drugs.

Andén et al. (1978) have postulated that R28935 lowers the blood pressure by a reduced influence of central noradrenaline due to blockade of postsynaptic α-receptors. Whether these receptors are to be found in the mentioned brain areas remains to be determined.

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