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AFFINITY OF VARIOUS LIGANDS FOR BENZODIAZEPINE RECEPTORS IN RAT CEREBELLUM AND HIPPOCAMPUS

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Abstract—The structure–affinity relationship of benzodiazepine receptor ligands for binding to their receptors was investigated by measuring the potency of 41 benzodiazepines or benzodiazepine analogues and of 9 non-benzodiazepines for inhibition of [^{3}H]flunitrazepam binding to cerebellar or hippocampal membranes. It was found that a chloro or a fluoro substitutent in position 2′ enhanced whereas substituents in position 3 and substituents larger than a methyl group in position 1 of the benzodiazepine ring system reduced the potency of benzodiazepines for inhibition of [^{3}H]flunitrazepam binding in cerebellum or hippocampus. In addition, several benzodiazepines could be identified which have a higher affinity for benzodiazepine receptors in cerebellum than for those in hippocampus. A selectivity of benzodiazepines for their receptors in cerebellum seems to be caused not only by certain substituents in position 1 but also by a chloro group in position 2′ and together with this substituent by a hydroxy group in position 3.

The discovery of specific high affinity binding sites for [^{3}H]diazepam or [^{3}H]flunitrazepam in brain membranes [1–3] has enormously stimulated research on the molecular mechanism of action of benzodiazepines. Thus, a close association of these binding sites with a GABA receptor, a chloride ion channel and several different drug binding sites has been demonstrated [4], and it is now generally assumed that the specific high affinity binding sites for [^{3}H]-diazepam or [^{3}H]flunitrazepam are the receptors by which the benzodiazepines exert their pharmacologically and clinically relevant actions [5].

Since all benzodiazepines investigated were able to displace [^{3}H]diazepam or [^{3}H]flunitrazepam with similar potency in different brain regions yielding Hill coefficients close to unity [6, 7], originally it has been assumed that there is only one type of benzodiazepine receptor in the brain. However, recently several compounds have been identified which seem to differentially interact with different benzodiazepine receptors. Thus, the triazolopyridazine Cl 218 872 [7], the methyl-(βCCM), ethyl-(βCCE) and propyl-(βCCP) esters of β-carboline-3-carboxylate [6] and very recently some benzodiazepines like quazepam, SCH 15 725 and SCH 23 324 [8] have shown to have a several times higher affinity for benzodiazepine receptors in cerebellum than for those in hippocampus and other brain regions. In addition, displacement of [^{3}H]flunitrazepam binding by these compounds yielded Hill coefficients close to unity in cerebellum but about 0.6–0.7 in hippocampus suggesting homogeneity and heterogeneity of benzodiazepine binding sites in cerebellum and hippocampus, respectively.

In the present study, 41 benzodiazepines or benzodiazepine analogues and 9 non-benzodiazepines which have high affinity for benzodiazepine receptors were investigated for their potency to displace [^{3}H]-flunitrazepam from cerebellar or hippocampal membranes. Several benzodiazepines could be identified which differentially interact with benzodiazepine receptors in cerebellum or hippocampus. In addition, several structural features could be identified which seem to enhance selectivity of benzodiazepines for their receptors in cerebellum.

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MATERIALS AND METHODS

Tissue from rat hippocampus or cerebellum was homogenized with an Ultra Turrax for 30 sec in 50 vol. ice-cold 50 mM Tris-citrate buffer, pH 7.1, and centrifuged for 10 min at 48,000 *g* at 0°. The pellets were washed five times by resuspension and recentrifugation in the same volume of ice-cold buffer and stored at −20° for at least 18 hr. After thawing, aliquots of the membrane suspension were pelleted and resuspended in 50 vol. of the same buffer.

Inhibition of [^{3}H]flunitrazepam binding by benzodiazepine receptor ligands was investigated by incubating 0.1 ml of membrane suspensions for 90 min at 0° with 2 nM [^{3}H]flunitrazepam (76.9 Ci/mmole, NEN) in the absence or presence of 2 μM diazepam or various concentrations of different benzodiazepine receptor ligands in a total of 1 ml of an incubation medium which contained 50 mM Tris–citrate buffer, pH 7.1, and 150 mM NaCl. The benzodiazepine receptor ligands were dissolved in absolute ethanol and diluted into the incubation medium immediately before the assay. Maximal ethanol concentration in the final incubation medium was 1% (v/v) ethanol, and it was demonstrated that this ethanol concentration did not influence specific high affinity binding of [^{3}H]flunitrazepam to brain membranes.

Table 1. Structure–affinity relationship of benzodiazepines for their receptors in cerebellum or hippocampus

No.	Compounds	Structure	R_7	R_2'	R_1	R_3	Cerebellum IC_{50} (nM)	Hippocampus IC_{50} (nM)	IC_{50} ratio Hip/Cb
1	Ro 5-2180	I	Cl	H	—H	H	23.0 ± 2.0	23.0 ± 1.0	1.00
2	Diazepam	I	Cl	H	$-CH_3$	H	12.8 ± 1.4	13.6 ± 4.2	1.06
3	Prazepam	I	Cl	H	$-CH_2-CH\langle^{CH_2}_{CH_2}$	H	418.0 ± 11.5	501.0 ± 26.0	1.20
4	Oxazepam	I	Cl	H	—H	OH	43.7 ± 7.4	38.3 ± 17.0	0.88
5	Temazepam	I	Cl	H	$-CH_3$	OH	33.5 ± 3.0	37.0 ± 1.0	1.10
6	Ox 320	I	Cl	H	$-CH_2-C\equiv CH$	OH	476.0 ± 20.0	616.0 ± 71.0	1.29
7	Ox 313	I	Cl	H	$-CH_2-CH_2-CN$	OH	230.0 ± 26.0	788.0 ± 191.0	3.43
8	Ox 326	I	Cl	H	$-CH_2-CH_2-C\langle^{NOH}_{NH_2}$	OH	992.0 ± 226.0	1863.0 ± 148.0	1.88
9	Ox 306	I	Cl	H	$-CH_2-CH_2-N\langle^{C_2H_5}_{C_2H_5}$	OH	1065.0 ± 50.0	990.0 ± 127.0	0.93
10	Ox 316	I	Cl	H	$-CH_2-CH(O)-CH_2$	OH	550.0 ± 70.0	850.0 ± 71.0	1.55
11	Ox 310	I	Cl	H	$-CH_2-CH_2-OCH_3$	OH	1400.0 ± 100.0	3000.0 ± 283.0	2.14
12	Ox 322	I	Cl	H	$-CH_2-CH_2-OCH_3$	$-OCOCH_3$	> 10,000	> 10,000	—
13	Ox 308	I	Cl	H	$-CH_2-CH_2-OCOCH_3$	OH	1650.0 ± 210.0	2250.0 ± 71.0	1.36
14	Ro 5-3027	I	Cl	Cl	—H	H	0.9 ± 0.2	1.6 ± 0.4	1.84
15	Lorazepam	I	Cl	Cl	—H	OH	1.1 ± 0.2	3.2 ± 0.7	3.02
16	Ox 353	I	Cl	Cl	$-CH_2-CH_2-CN$	OH	7.4 ± 2.0	38.9 ± 7.6	5.27
17	Ox 353 C	I	Cl	Cl	$-CH_2-CH_2-CN$	$-OCH_2CH_2CN$	> 10,000	> 10,000	—
18	Ro 5-3367	I	Cl	F	—H	H	2.8 ± 1.4	3.1 ± 1.3	1.13
19	SCH 15 725 2(C=O)	I	Cl	F	$-CH_2-CF_3$	H	13.9 ± 5.9	111.0 ± 33.0	8.00
20	Quazepam 2(C=S)	I	Cl	F	$-CH_2-CF_3$	H	29.7 ± 3.4	163.7 ± 18.4	5.50
21	Flurazepam	I	Cl	F	$-CH_2-CH_2-N\langle^{C_2H_2}_{C_2H_5}$	H	62.3 ± 12.5	69.3 ± 17.0	1.11
22	Ox 164 F	I	Cl	F	H	OH	6.2 ± 0.1	6.9 ± 1.6	1.11
23	SCH 23 324	I	Cl	F	$-CH_2-CF_3$	OH	30.9 ± 1.8	140.0 ± 5.6	4.50
24	Cinolazepam	I	Cl	F	$-CH_2-CH_2-CN$	OH	42.7 ± 7.5	132.7 ± 14.4	3.10
25	Ox 378	I	Cl	F	$-CH_2-CH_2-C\langle^{NOH}_{NH_2}$	OH	58.7 ± 18.0	101.3 ± 13.0	1.73
26	Ox 372	I	Cl	F	$-CH_2-CH_2-OCOCH_3$	OH	287.0 ± 10.0	446.0 ± 20.0	1.55
27	Ox 167 F	I	Cl	F	H	$-OCOCH_3$	28.5 ± 10.6	39.5 ± 14.8	1.39
28	Ox 373 C	I	Cl	F	$-CH_2CH_2CN$	$-OCH_2CH_2CN$	> 10,000	> 10,000	—

29	Nitrazepam	I	NO_2	H	H	H	24.0 ± 6.8	20.8 ± 3.0	0.87
30	Ox 164 NO_2	I	NO_2	H	H	OH	72.0 ± 5.7	66.5 ± 10.6	0.92
31	Clonazepam	I	NO_2	Cl	H	H	1.3 ± 0.1	2.8 ± 0.5	2.18
32	Flunitrazepam	I	NO_2	F	CH_3	H	2.9 ± 0.7	2.7 ± 0.1	0.92
33	Ro 11-6893	I	NO_2	F	CH_3	CH_3	106.7 ± 11.5	96.3 ± 12.0	0.90
34	Ro 11-6896	I	NO_2	F	CH_3	CH_3	13.0 ± 6.6	10.3 ± 4.7	0.79
35	Estazolam	II	Cl	H	CH_3	H	21.1 ± 1.9	21.4 ± 3.6	1.00
36	Alprazolam	II	Cl	H	CH_3	H	14.3 ± 4.5	13.3 ± 3.2	0.93
37	Triazolam	II	Cl	F	CH_3	H	0.4 ± 0.2	0.5 ± 0.1	1.24
38	Midazolam	III	Cl	F	CH_3	H	2.0 ± 0.3	2.5 ± 0.3	1.21
39	Premazepam	IV	—	—	—	—	860.0 ± 50.0	902.0 ± 36.0	1.04
40	Clobazam	VI	—	—	—	—	518.0 ± 125.0	530.0 ± 142.0	1.02
41	Ro 15-1788	V	—	—	—	—	1.0 ± 0.5	1.3 ± 0.6	1.24

Membranes from rat cerebellum or hippocampus were incubated with 2 nM [^{3}H]flunitrazepam in the absence or presence of 2 μM diazepam and various concentrations of benzodiazepines to be tested as described in Materials and Methods. The samples were then filtered under vacuum through Whatman GF/B filters and radioactivity on the filters was determined by liquid scintillation counting. The concentrations resulting in half maximal inhibition of specific [^{3}H]-flunitrazepam binding (IC_{50}) are mean values ± S.D. from 3 to 5 independent experiments performed in duplicate. K_d values for [^{3}H]flunitrazepam binding were 2.9 ± 0.3 nM and 2.0 ± 0.2 nM (means ± S.D., N = 3) for membranes from cerebellum or hippocampus, respectively. "Structure" refers to compound-structures I–VI shown in Fig. 1. In additional experiments, the inhibition of [^{3}H]flunitrazepam binding by quazepam, SCH 15 725, Cl 218 872 or βCCE was investigated at 37°. Although IC_{50} values of these compounds were significantly larger at this temperature, the differences in IC_{50} values between cerebellum and hippocampus were again demonstrated for all these compounds at this more physiological temperature.

The samples were then filtered under vacuum through Whatman GF/B filters and immediately washed twice with a buffer containing 50 mM Tris–citrate, pH 7.1. The radioactivity on the filters was measured by liquid scintillation counting. Non specific binding was determined in the presence of 2 μM diazepam and was subtracted from total binding to give specific binding.

Compounds investigated in this study were obtained from Dr. Braestrup, Ferrosan, Denmark (βCCM, βCCE, βCCP, DMCM); Dr. Mauracher, Essex Chemie, Switzerland (Quazepam, SCH 15 725, SCH 23 324); Dr. Möhler, Hoffman La Roche, Switzerland (Diazepam, Oxazepam, Flurazepam, Nitrazepam, Flunitrazepam, Clonazepam, Ro 5-2180, Ro 5-3027, Ro 5-3367, Ro 11-6893, Ro 11-6896, Ro 15-1788); Dr. Blanchard, Rohne-Poulenc, France (Zopliclone); Dr. Nitsche, Gerot, Vienna (Cinolazepam, Ox 164 F, Ox 164 NO_2, Ox 306, Ox 308, Ox 310, Ox 313, Ox 314, Ox 316, Ox 320, Ox 326, Ox 353, Ox 378, Ox 372); Wyeth Laboratories, U.S.A. (Lorazepam); Takeda Chemical Industries, Japan (Estazolam); Ciba Geigy, U.S.A. (CGS 8216, CGS 9896, CGS 9895); Farmitalia Carlo Erba, Italy (Temazepam); Upjohn, U.S.A. (Alprazolam, Triazolam).

RESULTS

The influence of the molecular structure of benzodiazepines on the potency of 41 different benzodiazepines as inhibitors of [^{3}H]flunitrazepam binding was investigated in membranes from rat cerebellum or hippocampus. In agreement with previous reports [9, 10], compounds with a chloro substituent in position 7 (Fig. 1, compound I) have an affinity for benzodiazepine receptors similar to compounds with a nitro substituent in the same position (compare compounds 1 and 29, 14 and 31, Table 1).

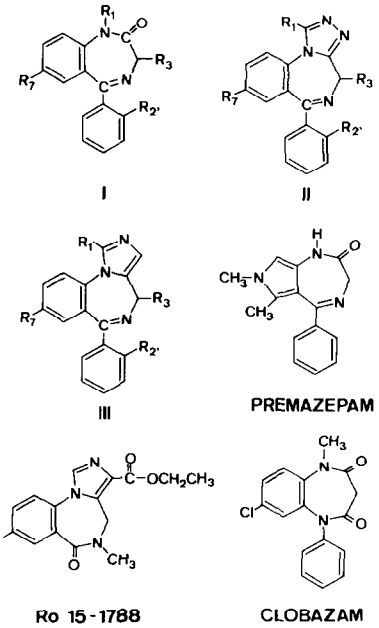

Fig. 1. Structural formula of different benzodiazepines.

Both a chloro substituent (compare compounds 1 and 14, 4 and 15, 7 and 16, 29 and 31, Table 1) and a fluoro substituent (compare compounds 1 and 18, 4 and 22, 7 and 24, 8 and 25, and 13 and 26, Table 1) in position 2′ (Fig. 1, compound I) enhance the affinity of benzodiazepines for their receptor, however the enhancement of a chloro substituent in this position is larger than that of a fluoro substituent (compare compounds 14 and 18, 15 and 22, 16 and 24, Table 1). In addition, a chloro substituent in position 2′ enhanced the affinity for receptors in cerebellum more strongly than the affinity for receptors in hippocampus, resulting in a differential effect of the respective compounds on receptors in cerebellum and hippocampus (compare compounds 1 and 14, 4 and 15, 7 and 16, and 29 and 31, Table 1). This differential effect of the benzodiazepine ligands for their receptors in cerebellum and hippocampus caused by a chloro substituent in position 2′ seems to be further enhanced by a hydroxy substituent in position 3 (Fig. 1, compound I) of the benzodiazepine ring system (compare compounds 1 and 4 with compounds 14 and 15, Table 1). In contrast, introduction of a fluoro substituent into position 2′ did not cause any differential affinity for benzodiazepine receptors in cerebellum and hippocampus (compare compounds 1 and 18, 7 and 24, 8 and 25, 13 and 26). In addition, when position 2′ is occupied by a fluoro instead of a chloro substituent, a hydroxy group in position 3 seems not to cause (Table 1, compounds 18 and 22) or even reduce (Table 1, compounds 19 and 23) a differential affinity of benzodiazepines for their receptors in cerebellum or hippocampus.

As shown in Table 1, a methyl group in position 1 of the benzodiazepine ring system (Fig. 1, compound I) slightly enhanced (compare compounds 1 and 2, and 4 and 5, Table 1) whereas larger substituents reduced the affinity of benzodiazepines for their receptors (compare compounds 1 and 3, 4 and 6–13, 15 and 16, 18 and 19–21, and compounds 22 and 23–26, Table 1). However, certain substituents in position 1 reduced the affinity of benzodiazepines for their receptors less strongly in cerebellum than in hippocampus, resulting in a differential potency of benzodiazepines for inhibition of [^{3}H]flunitrazepam binding in these brain regions. Thus, for instance, a cyanoethyl group (compare compounds 4 and 7, 15 and 16, 22 and 24, Table 1) or a trifluoroethyl group [8] (compare compounds 18 and 19, 22 and 23, Table 1) in position 1 of the benzodiazepine ring system resulted in a severalfold higher affinity for benzodiazepine receptors in cerebellum than for those in hippocampus. In contrast, a $-CH_2CH_2-O-CH_3$ group (compare compounds 4 and 11, Table 1) or a $-CH_2-CH_2-C(NH_2)=NOH$ group (compare compounds 4 and 8; 22 and 25, Table 1) only very weakly differentiated between receptors in cerebellum and hippocampus.

The replacement of the carbonyl group in position 2 of the benzodiazepine ring system by a thiocarbonyl group reduced the affinity of the benzodiazepines for the benzodiazepine receptor (compare compounds 19 and 20, Table 1) [8]. In addition, substituents in position 3 of the benzodiazepine ring system reduced the affinity of benzodiazepines for their receptors (see compounds 1 and 4, 2 and 5, 14 and 15, 18 and 22, 19 and 23, 18 and 27, 28; 29 and 30, and compounds 32 and 33, 34, Table 1), and the reduction in affinity of benzodiazepines increased with the size of the substituent in position 3 (compare compounds 11 and 12, 16 and 17, 22 and 27, 22 and 28, 32 and 33, 34, Table 1). In agreement with previous reports [1, 10, 11], the 3S stereoisomer Ro 11-6896 had a higher affinity for benzodiazepine receptors than the 3R stereoisomer Ro 11-6893 (compounds 33 and 34).

The fusion of an additional heterocyclic ring to the benzodiazepine ring system (Fig. 1, compounds II and III) did not change the affinity of the original benzodiazepine (see compounds 1 and 35, Table 1). In addition, a methyl substituent in this new ring system enhanced the affinity of these compounds in a way similar to that of a methyl group in position 1 of the original benzodiazepine (compare compounds 1 and 2 with 35 and 36). In contrast, a pyrrolo ring (Fig. 1, IV) instead of the benzolo ring of the benzodiazepine significantly reduced the affinity for benzodiazepine receptors (compare compounds 1 and 39) possibly because of the lack in premazepam of an electrophilic substituent in a position corresponding to position 7 of the benzodiazepine ring system. Interestingly the potency for inhibition of [^{3}H]flunitrazepam binding of compounds III and V in Fig. 1 were of a similar order of magnitude (see compounds 38 and 41, Table 1). The 1,5-benzodiazepine clobazam, however (Fig. 1, VI), had a significantly reduced potency for [^{3}H]flunitrazepam binding sites in cerebellum and hippocampus (see compounds 2 and 40, Table 1).

In order to compare the selectivity of the cyanoethyl benzodiazepines cinolazepam and Ox 353 (compounds 16 and 24) and the trifluoroethyl benzodiazepines quazepam, SCH 15 725 and SCH 23 324 (compound 19, 20, 23) with that of non benzodiazepine receptor ligands, the potency of several non benzodiazepines (Fig. 2) for displacement of [^{3}H]flunitrazepam binding was investigated in cerebellum and hippocampus. In agreement with previous reports Cl 218 872 [7], βCCM, βCCE and βCCP [6] had a higher affinity for benzodiazepine receptors in cerebellum than for those in hippocampus (Table 2). In contrast, DMCM, a con-

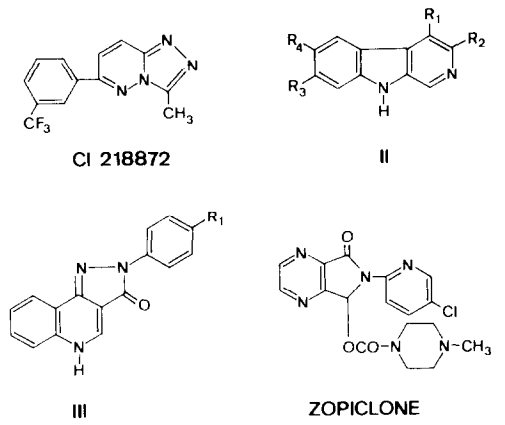

Fig. 2. Structural formula of various benzodiazepine receptor ligands.

Table 2. Potency of various benzodiazepine receptor ligands as inhibitors of specific [^{3}H]flunitrazepam binding to membranes from cerebellum or hippocampus

No.	Compounds	Structure	R_1	R_2	R_3,R_4	Cerebellum IC_{50} (nM)	Hippocampus IC_{50} (nM)	IC_{50} ratio Hip/Cb
1	Cl 218 872	I	—	—	—	190.3 ± 26.8	1296.7 ± 105.0	6.81
2	βCCM	II	H	—$COOCH_3$	H	0.8 ± 0.1	4.0 ± 1.0	5.19
3	βCCE	II	H	—$COOC_2H_5$	H	1.1 ± 0.3	4.8 ± 0.8	4.44
4	βCCP	II	H	—$COOC_3H_7$	H	2.1 ± 0.9	13.2 ± 2.9	6.17
5	DMCM	II	—C_2H_5	—$COOCH_3$	—OCH_3	5.1 ± 1.4	5.7 ± 1.4	1.12
6	CGS 8216	III	H	—	—	0.067 ± 0.018	0.152 ± 0.028	2.28
7	CGS 9895	III	—OCH_3	—	—	0.087 ± 0.011	0.188 ± 0.039	2.16
8	CGS 9896	III	Cl	—	—	0.129 ± 0.065	0.372 ± 0.049	2.89
9	Zopiclone	IV	—	—	—	71.7 ± 12.9	129.3 ± 21.0	1.80

Membranes from rat cerebellum or hippocampus were incubated with 2 nM [^{3}H]flunitrazepam in the absence or presence of 2 μM diazepam and various concentrations of benzodiazepine receptor ligands to be tested as described in Materials and Methods. The samples were then filtered under vacuum through Whatman GF/B filters and radioactivity on the filters was determined by liquid scintillation counting. The concentrations resulting in half maximal inhibition of specific [^{3}H]flunitrazepam binding (IC_{50}) are mean values ± S.D. from 3 to 5 independent experiments performed in duplicate. "Structure" refers to compound-structures I–IV shown in Fig. 2.

vulsive β-carboline did not show any differential affinity for benzodiazepine receptors in cerebellum and hippocampus under the experimental conditions used. This is in contrast to the slightly higher affinity of DMCM for benzodiazepine receptors in hippocampus observed under chloride free conditions [12, (Eichinger and Sieghart, unpublished observations)]. Although the pyrazoloquinolines CGS 8216, CGS 9895 and CGS 9896 [13] are about twice as potent for the displacement of [^{3}H]flunitrazepam in cerebellum than for than in hippocampus, the selectivity of these compounds is smaller than that of Cl 218 872, βCCM, βCCE or βCCP. Similarly, zopliclone [14], a cyclopyrrolone, exhibited a weak but significant selectivity for benzodiazepine receptors in cerebellum.

DISCUSSION

In the present study, the structure–affinity relationship of 41 benzodiazepines and 9 non-benzodiazepines for benzodiazepine receptors in cerebellum and hippocampus was investigated. In agreement with previous reports [9, 10], it was demonstrated that a chloro or fluoro substituent in the 2′ position enhanced whereas substituents in position 3 reduced the affinity of benzodiazepines for their receptors. Similarly, substituents larger than a methyl group in position 1 generally reduced the affinity of benzodiazepines for their receptors [10]. However, some substituents in position 1 were identified which caused a differential reduction of affinity for benzodiazepine receptors in cerebellum and hippocampus. Thus, as reported in a previous paper [8], a selectivity of the trifluoroethyl benzodiazepines quazepam, SCH 15 725 and SCH 23 324 for benzodiazepine receptors in cerebellum was observed. In addition, it was found that other substituents in position 1, like a cyanoethyl group, a $CH_2CH_2OCH_3$ group and a CH_2CH_2—$C(NH_2)$=NOH group too caused some selectivity for benzodiazepine receptors in cerebellum. Furthermore, from the results of the present study, some general principles seem to evolve which govern the selectivity of benzodiazepines for their receptors in cerebellum. Thus, it can be concluded that not only certain substituents in position 1 but also a chloro group in position 2′ and together with this substituent a hydroxy group in position 3 can enhance the selectivity of benzodiazepines for their receptors in cerebellum. Although there is only a limited amount of data available at the moment, the results of the present study suggest that a benzodiazepine analogue of SCH 15 725 or SCH 23 324 which has a chloro group instead of a fluoro group in position 2′, should exhibit a selectivity for cerebellar benzodiazepine receptors even higher than SCH 15 725.

In addition, the present results support previous evidence for a heterogeneity of benzodiazepine receptors in rat brain [6, 7, 8, 15, 17, 18]. Since the potency of selective and non selective benzodiazepine receptor ligands for inhibition of [^{3}H]-flunitrazepam binding was investigated in the same membrane preparations isolated from rat cerebellum or hippocampus the differential potencies of only some ligands observed in these different brain regions cannot be attributed to differences in the isolation of membranes or in the state of modulation of benzodiazepine receptors by GABA or other endogenous factors [4, 5] in different brain regions. Thus, it has to be concluded that benzodiazepine receptors in cerebellum have a different way of interaction with some but not with other benzodiazepines or benzodiazepine receptor ligands than benzodiazepine receptors in hippocampus. In addition, the fact that Cl 218 872 [8, 15], βCCP [15], βCCE (Table 1), quazepam and SCH 15 725 [8] not only at 0° but also at 37° were able to differentiate between benzodiazepine receptors in cerebellum and hippocampus indicates that the observed differences in the binding properties of benzodiazepine receptors in cerebellum and hippocampus are not due to a low temperature artifact.

When data from all the benzodiazepine receptor ligands shown in Tables 1 and 2 exhibiting an at least 3-fold higher potency for inhibition of [^{3}H]-

flunitrazepam binding to membranes from cerebellum than for those in hippocampus were subjected to Hill analysis, Hill coefficients were obtained which were close to unity in cerebellar and around 0.6–0.8 in hippocampal membranes supporting a homogeneity and heterogeneity of receptors in cerebellum and hippocampus, respectively. However, in agreement with previous results [15, 16], Hill coefficients obtained from the study of inhibition by these compounds of [^{3}H]flunitrazepam binding to membranes from hippocampus at 37° were more close to unity than those obtained in studies at 0°. Thus, the present results cannot be used to support an intraregional heterogeneity of benzodiazepine receptors in hippocampus in addition to an interregional heterogeneity of benzodiazepine receptors in cerebellum and hippocampus. However, the recent demonstration that CL 218 872, βCCE and SCH 15 725 not only at 0° [18] but also at 37° were able to differentially inhibit irreversible binding of [^{3}H]flunitrazepam to different benzodiazepine binding proteins in hippocampal membranes (A. Eichinger and W. Sieghart, *J. Neurochem.* accepted for publication), seems to support an intraregional heterogeneity of benzodiazepine receptors in hippocampal membranes.

Whereas several benzodiazepines have now been identified which have a higher affinity for benzodiazepine receptors in cerebellum than for those in hippocampus thus far no compound has been identified which has a significantly higher affinity for benzodiazepine receptors in hippocampus than for those in cerebellum. Although DMCM has been reported [12] to preferentially interact with a type II benzodiazepine receptor, the slightly higher affinity of this compound for receptors in hippocampus compared with those from cerebellum in the absence of chloride ions [12] disappears in the presence of chloride ions (Table 2, Eichinger and Sieghart, unpublished results). Thus, additional studies have to be performed to identify compounds which selectively interact with benzodiazepine receptors in hippocampal membranes.

The physiological significance of different benzodiazepine receptors is not known. As shown in Tables 1 and 2, the selectivity of the various differential ligands for benzodiazepine receptors in cerebellum is not very large. Therefore it is to be expected that at the usual therapeutical doses these compounds will interact more or less with all different benzodiazepine receptors. In addition, the selective benzodiazepines will largely loose their selectivity for the receptors enriched in cerebellum when the substituent in position 1 is removed by metabolism of the benzodiazepines in the body. Therefore, it will be difficult to relate a possible different action of these compounds to their selective interaction with the benzodiazepine receptor subtype enriched in cerebellum. Further experiments have to be performed to find metabolically stable compounds with even higher differential affinity for the various benzodiazepine receptors in order to clarify the function of these receptors.

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