^{3}H-Diazepam binding sites in roman high- and low-avoidance rats

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Summary. The binding of ^{3}H-diazepam to membrane benzodiazepine receptors was examined in 2 psychogenetically selected lines of rats, which differ according to the selection criterion in avoidance behaviour (RHA/Verh > RLA/Verh) and, in addition, in emotionality (RHA/Verh < RLA/Verh). RHA/Verh rats tended to show higher specific diazepam binding in all CNS-subregions when compared with RLA/Verh animals. Significant differences were found in the cortex, striatum, hippocampus, thalamic region and pons-medulla. These results reinforce the contention that a system involving benzodiazepine receptors may play a role in emotional behaviour.

Several investigators have recently reported the existence of binding sites for ^{3}H-diazepam which meet the criteria established for identifying physiological receptors, e.g. rapid, reversible, stereospecific and saturable binding[2,3]. In both in vivo and in vitro experiments, diazepam binding sites have been found in the brain (with differential regional distribution) as well as in several peripheral tissues[4,5].

Robertson[6] has reported that 2 strains of rats, bred for high and low fearfulness (Maudsley reactive (MR) and Maudsley nonreactive [MNR]) have significantly different densities of central benzodiazepine binding sites. These 2 strains differ in their emotionality, and the suggestion was made that ^{3}H-diazepam binding may provide a neurochemical parameter for the measurement of emotional behaviour[6]. Similar findings in inbred strains of mice have confirmed the idea that a lower emotionality is connected with an increased ^{3}H-diazepam binding[7].

Roman high-avoidance rats (RHA/Verh) and Roman low-avoidance rats (RLA/Verh) show several behavioural parallels to the MNR and MR strains, respectively[8]. Open-field results for the 2 Roman lines were interpreted in terms of a reduced emotionality for RHA/Verh rats compared to RLA/Verh animals[9,10].

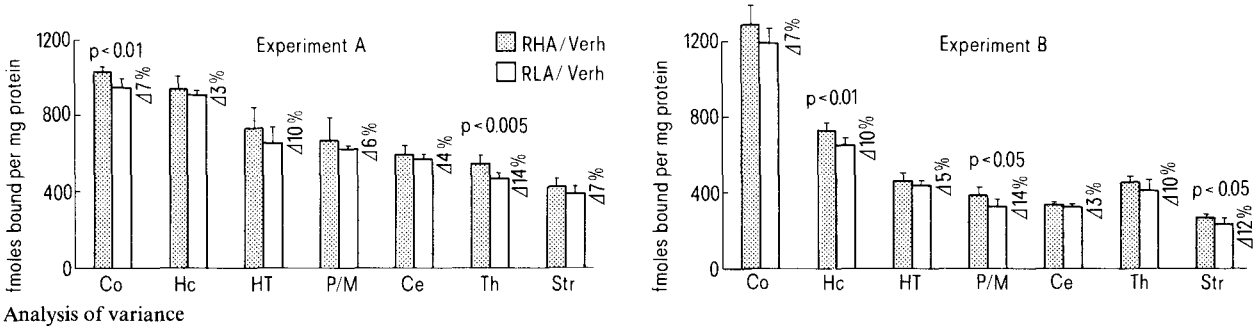
We therefore decided to test Robertson's hypothesis using RHA/Verh and RLA/Verh rats by measuring ^{3}H-diazepam binding in several brain regions of the 2 Roman lines.

Methods. Roman high-avoidance rats (RHA/Verh), bred at the Behavioural Science Institute ETH in Zürich for rapid acquisition of 2-way avoidance, and Roman low-avoidance rats (RLA/Verh), bred at the same institute for poor acquisition, were used. After a 3-week adaptation period to the new housing conditions (reversed light/dark cycle), rats were individually housed for 24 h prior to being killed by decapitation (between 13.00 and 16.00 h, in the middle of the dark phase).

Brains from 8-month-old RHA/Verh and RLA/Verh male rats were dissected on ice according to the method of Glowinski and Iversen[11] into cortex, striatum, cerebellum, hippocampus, pons-medulla, hypothalamus and the thalamic region. Samples were frozen on dry ice and stored at -70 °C.

Binding assays were carried out according to well-established methods. Briefly, tissues were homogenized (Polytron, type PT 10-35, position 5 for 30 sec) at a tissue concentration of 12.5 mg/ml Krebs-Ringer buffer (NaCl 133 mM, KCl 5 mM, $CaCl_2$ 2.7 mM, $MgSO_4$ 1.2 mM, Tris 10 mM and glucose 10 mM, adjusted to pH 7.4 with HCl). The homogenate was centrifuged at $33{,}000 \times g$ for 10 min and the resulting pellet was rehomogenized (15 sec) in the same volume of buffer. After repeated additional washing, 500 µl homogenate (corresponding to 6.25 mg original wet wt) were incubated for 15 min on ice with 3 nM ^{3}H-methyl-diazepam (sp.act. 83.5 Ci/mmole, New England Nuclear [NEN]) in a total volume of 2 ml. Nonspecific binding was determined using 10^{-5} M cold diazepam (a gift from Dr Möhler, Hoffmann La Roche, Basel). The incubation was stopped by filtration under vacuum through Whatman GF/B glass fiber filters and the membrane fragments rapidly washed with 2×5 ml cold buffer. Filters were counted in 10 ml Instagel (Packard).

Determinations were carried out in triplicate and, after



Analysis of variance

Co	Hc	HT	P/M	Ce	Th	Str
F(1.20)	F(1.20)	F(1.20)	F(1.20)	F(1.20)	F(1.20)	F(1.20)
= 9.8	= 8.6	= 2.7	= 4.6	= 1.9	= 13.9	= 6.6
$p < 0.01$	$p < 0.01$	NS	$p < 0.05$	NS	$p < 0.001$	$p < 0.05$

(Inter-strain effects of 2 experiments were summed up by ANOVA [see 'methods']).

Regional analysis of specific ^{3}H-diazepam binding in roman high-avoidance (RHA/Verh) and roman low-avoidance (RLA/Verh) male rats. Specific binding at 3 nM ^{3}H-diazepam is expressed as fmole diazepam bound per mg protein. Experiments A and B were carried out separately using 6 animals of both rat lines each time; each column represents the mean ± SD for 6 animals. Statistical comparisons within each experiment were carried out by using Student's t-test (2-tailed). An additional comparison (see bottom of the figure) was carried out by using 2 way analysis of variance (summing up the inter-strain effects of the 2 separate experiments). Abbreviations: Co. cortex; Hc. hippocampus; HT. hypothalamus; P/M. pons-medulla; Th. thalamic region; Ce. cerebellum; Str. striatum.

randomisation, corresponding areas from the 2 rat lines were analyzed at the same time.

Protein was determined according to the method of Lowry[12]. Specific binding was measured during 2 independent experiments and these results are shown separately for both experiments. Because of inhomogeneous variances, data were transformed in to natural logarithms prior to statistical evaluation. Then the significance of differences betwen RHA/Verh and RLA/Verh rats within each experiment was assessed by Student's t-test (2-tailed); since inter-experimental variations were observed, transformed data were further statistically compared by 2 way analysis of variance (summing up the inter-strain effects of the 2 experiments [fig., bottom]).

Results. Specific ^{3}H-diazepam binding in RLA/Verh rats is significantly reduced in the cortex and thalamic region in experiment A, and in the striatum, hippocampus and pons-medulla in experiment B when compared with RHA/Verh animals. In both experiments in all other regions a trend in the same direction (RLA/Verh < RHA/Verh) was observed, but these differences did not reach a significant level (fig.). Overall inter-strain effects, as assessed by analysis of variance, reached a significant level in the cortex, striatum, hippocampus, thalamic region and pons-medulla. Levels of binding showed a merely equal regional distribution in both experiments (cortex > hippocampus > hypothalamus, pons-medulla, cerebellum, thalamic region > striatum).

Inter-experimental variations, which were observed in all regions, remain obscure (seasonal variations?); however, in none of the regions was any interaction between inter-strain and inter-experimental effects indicated by the analysis of variance, so it was assumed that inter-strain differences are independent of the absolute levels of binding.

Due to the limited number of animals at our disposal, Scatchard analyses were only possible on pooled tissues from each rat line for the cortex, hippocampus and pons-medulla (experiment B). In all 3 regions a decreased binding capacity (reductions between 16 and 21%) and a reduction in the dissociation constant (between 20 and 32%) was observable in RLA/Verh rats when compared with the RHA/Verh counterparts. However, further determinations are required before conclusive interpretations of the differences in B_{max}- and K_D-values between these 2 rat lines are possible.

Discussion. Differences in ^{3}H-diazepam binding between 2 rat lines differing in emotional behaviour, as reported in this paper, confirm Robertson's hypothesis[6] that a lower emotionality is connected with higher specific binding.

Significant differences in ^{3}H-diazepam binding between RHA/Verh and RLA/Verh rats were only detectable in some central regions (cortex, striatum, hippocampus, thalamic region and pons-medulla). However, we found in the other regions, as did Robertson[6] in his 2 strains, non-significant reductions as big as or even larger than those which had reached a significant level.

The degree of difference between the 2 Roman lines is slightly smaller (3–14%) than between MNR and MR animals (8–20%). It is interesting to note that we found one of the smallest differences in the cerebellum, as did Robertson in his animals[6]. In our experiments we found the largest percentage differences in ^{3}H-diazepam binding in the striatum, the pons-medulla and the thalamic region, exactly the 3 regions for which Robertson described the biggest differences. Based on the present binding study in Roman animals and on those reported by Robertson for MR and MNR rats, the postulated similarities between the Roman lines and the Maudsley strains[8] are further supported.

The present results could also be in accordance with the only previous report of differences in ^{3}H-diazepam binding in rats bred for defined conditional avoidance responding. Some unpublished data of Broadhurst were reported recently in a paper by Braestrup[13]: 'Small (8–11%, $p < 0.05$) differences were found in Roman rats between one group which was selected and inbred for high conditional avoidance and another group of nonselected inbred control animals from the same strain'. The direction of change was not clearly described and information about the tissue used for the determination was lacking.

Braestrup[13] has recently reported that benzodiazepine binding is affected by some experimental stresses. Therefore, differences in ^{3}H-diazepam binding between RHA/Verh and RLA/Verh rats could possibly be due to a different stress level of the 2 selected lines. However, similar plasma levels of stress hormones in undisturbed RHA/Verh and RLA/Verh rats[10] make such an explanation for the different ^{3}H-diazepam binding rather unlikely.

Under the washing conditions used for the membrane fragments in the present experiments, it is possible that differences in endogeneous GABA-levels between the 2 rat lines could account for the apparent differences in ^{3}H-diazepam binding, as it has been shown that GABA and its analogue muscimol enhance ^{3}H-diazepam binding[15]. Unfortunately, GABA levels in these 2 lines have never, to our knowledge, been determined, although Rick[14] has reported that GABA production in the cerebral cortex was higher for RHA.

Finally, at present it is not clear whether these differences in ^{3}H-diazepam binding, even if they are statistically significant, are of biological relevance. Therefore, further experiments should test a) whether structural differences (different membrane compositions) between RHA/Verh and RLA/Verh rats could account for the binding differences or b) whether different amounts of spare ^{3}H-diazepam binding sites could be responsible for such dissimilarities. Additionally, other binding sites should be tested to determine whether the difference between RHA/Verh and RLA/Verh rats is specific for ^{3}H-diazepam binding.

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