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Increased Benzodiazepine Receptor Binding in Rat Frontal Cortex Following Acute Forced Swimming in Cold Water

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Summary

Increased benzodiazepine receptor binding was observed in rat frontal cortex following acute forced swimming in cold water using ^3H -diazepam and ^3H -Ro15-1788 as radioligands. K_D showed no difference on either radioligands, but B_{max} indicated in the stress group a statistically significant increase in both ligands. However in other regions of the brain such as amygdala, hypothalamus and hippocampus, no significant changes were observed. In case of using ^3H - βCCE as radioligand, no changes were observed in both K_D and B_{max} in frontal cortex.

Rapid increase of benzodiazepine receptor binding might be brought by production of free, unoccupied receptors in response to acute stresses. Different profile between each radioligands might be come from presence or absence of action of these ligands to chloride ion channel.

Introduction

A better understanding of the neurobiological basis of anxiety produced by stress would be beneficial in developing more specific agents. Benzodiazepines probably exert their pharmacological and clinical effects through interaction with specific benzodiazepine receptors (BDZ-R) in the central nervous system (Brasestrup *et al.*, 1977 : Möhler and Okada, 1977 : Speth *et al.*, 1978).

Concerning the effect of stress on the central neurotransmitter mechanism, a role of BDZ-R seems to deserve proper attention. However, only few reports have so far been available. We have therefore subjected rats to a stress and investigated some characteristics of brain BDZ-R. Soublie *et al.* (1980) reported an increase of BDZ-R binding in frontal cortex of rat after an acute stress of swimming in cold water. In the present study, We have designed same kind of experiment to confirm their findings with a few modifications as follows.

Firstly, Soublie *et al.* (1980) used the rats put in water at 25°C as controls, assuming the difference of water temperature between 5°C and 25°C as a stressor. However, since forced swimming in warm water can itself be a considerable stress, we used the rats not subjected to

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swimming as controls.

Secondly, after observing an increase in specific binding of BDZ-R in frontal cortex (N=8), they performed a saturation experiment in one rat only, presuming that such increase is caused by a change of B_{max}. We performed a series of saturation experiments with a sufficient number of rats for statistical analysis of K_D and B_{max}.

Thirdly, they used ³H-flunitrazepam as radioligand. We employed ³H-diazepam, a typical agonist ligand of BDZ-R, ³H-Ro15-1788, an antagonist ligand, and also ³H-ethyl β -carboline-3-carboxylate (³H- β CCE), an inverse agonist ligand for the confirmation of findings.

Materials and Methods

Male rats of Wistar strain weighing 250–300 g were housed 4–6 animals per cage with *ad lib* access to food and water under alternate 12 hr light and dark periods. Acute cold swimming stress has been performed by putting in water at 5°C for 3 minutes. The rats were decapitated immediately after forced swimming. The brain was removed immediately, and according to the method of Heffner (1980) using an aluminum cutting block, frontal cortex, hypothalamus, amygdala and hippocampus were cut out. The specimens were immediately frozen in acetone dry ice and preserved at –80°C until assay.

This tissue was thawed and homogenized in 10 vol of ice cold 0.32M sucrose using a glass homogenizer fitted with a teflon paste (8 full passes). The crude homogenate was centrifuged at 1000 $\times$ g for 10 min and the resulting supernatant was centrifuged at 30000 $\times$ g for 30 min. The pellet was then resuspended in 20 vol of 25 mM Tris-HCl buffer, pH 7.3, using a Polytron homogenizer and centrifuged at 30000 $\times$ g for 20 min. This procedure was repeated twice. The final washed P₂ membrane fraction was used for binding assay.

The binding of ³H-diazepam (methyl-³H, 86.6 Ci/mmol, NEN) was carried out in accordance with the method of Möhler and Okada (1977). The binding was performed by incubating 0.2–0.5 mg protein, as determined by the method of Lowy et al. (1951) in 1 ml of 15mM Tris-Krebs buffer, pH 7.4, for 15 min at 0°C, using a range of 1.0–10 nM ³H-diazepam. Specific binding was calculated as the difference between samples with 1 μ M diazepam added and represented 90–95% of the total binding.

The binding of ³H-Ro15-1788 (N-methyl-³H, 87.0 Ci/mmol, NEN) was performed using the technique of Möhler *et al.* (1981). Briefly, samples containing 0.2–0.5 mg protein were suspended in 1 ml of 15 mM Tris-Krebs buffer, pH 7.4. The incubation, at 0°C for 60 min, was initiated by the addition of the membranes using a range of 0.25–1.25 nM ³H-Ro15-1788. To determine specific binding, parallel incubations were carried out in the presence of 1 μ M Ro15-1788 (a kind gift from Dr. Toshikazu Okada, Nippon-Roche Research Center) and represented 90–95% of the total.

The binding of ³H- β CCE (ethyl-2-³H, 80.6 Ci/mmol, NEN) was performed using the

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method of Marangos *et al.* (1981). Briefly, samples containing 0.2–0.5 mg protein were suspended in 1 ml of 15 mM Tris-chloride buffer, pH 7.3. The incubation, at 0°C for 15 min, was initiated by the addition of the membranes using a range of 0.2–2.0 nM ^3H - β CCE. To determine specific binding, parallel incubations were carried out in the presence of 3 μM diazepam and represented 90–95% of the total binding.

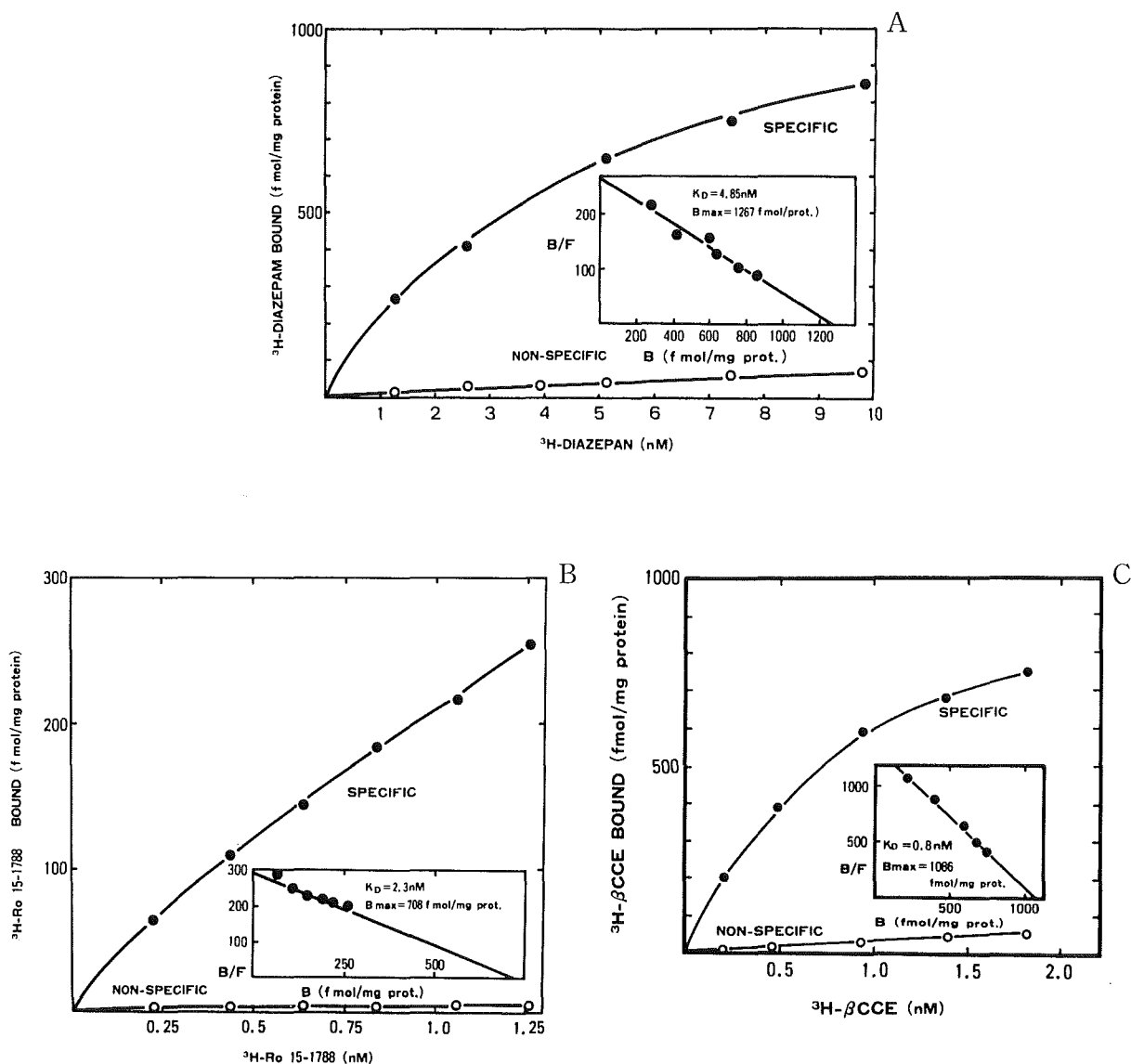


Fig. 1 Saturation of specifically bound ^3H -diazepam (A), ^3H -Ro15-1788(B) and ^3H - β CCE(C) in rat frontal cortex.

In all cases, binding was terminated by filtration under vacuum through GF/B Whatman filters, with 3 washes of 5 ml each with ice-cold buffer. The filters were dried in room temperature and counted in 7 ml of scintillation solution in a scintillation spectrometer. Fig. 1 shows typical saturation experiment in rat frontal cortex using ^3H -diazepam, ^3H -Ro15-1788 and ^3H - β CCE.

The significance of differences between control and stressed groups was calculated using Student's *t*-test.

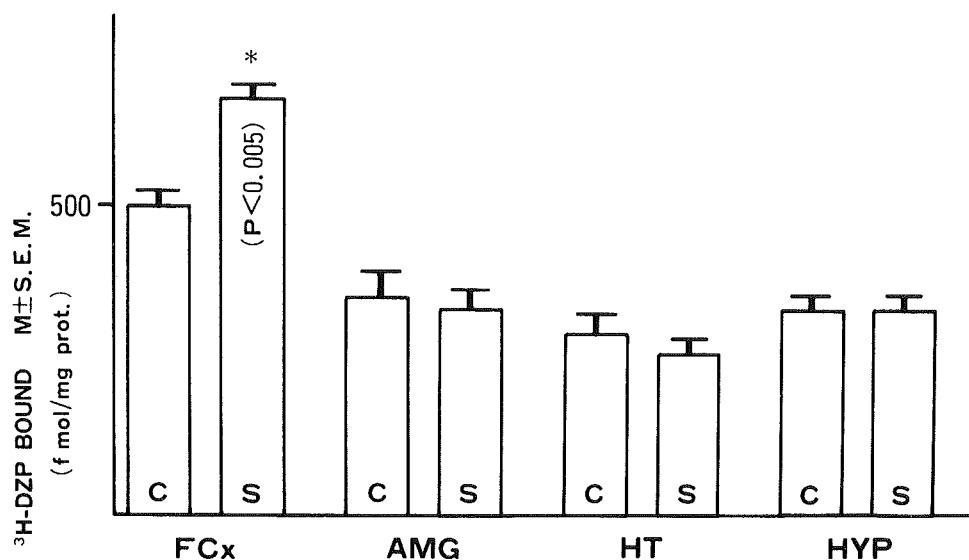


Fig. 2 Effects of acute forced swimming in cold water on ^3H -diazepam binding of rat brain.

Each vertical bar represents mean $\pm$ S. E. of ^3H -diazepam binding at 2 nM in four portions of rat brain (N=5)

Abbreviations in the figure : FCx, frontal cortex : AMG, amygdala : HT, hypothalamus : HYP, hippocampus : DZP, diazepam.

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Table 1 . Saturation experiments of ^3H -diazepam (A), ^3H -Ro15-1788(B) and ^3H - βCCE (C) of frontal cortex in control and stressed rats.

Each value represents mean $\pm$ S. E. * : $P < 0.025$.

	N	K_D (nM)	Bmax (fmol/mg prot.)	A
CONTROL	5	4.11 $\pm$ 1.17	1318 $\pm$ 132	
STRESSED	5	4.32 $\pm$ 1.18	1747 $\pm$ 100*	

	N	K_D (nM)	Bmax (fmol/mg prot.)	B
CONTROL	7	2.1 $\pm$ 0.6	794 $\pm$ 62	
STRESSED	8	1.9 $\pm$ 0.5	987 $\pm$ 39*	

	N	K_D (nM)	Bmax (fmol/mg prot.)	C
CONTROL	7	0.64 $\pm$ 0.06	992 $\pm$ 42	
STRESSED	7	0.54 $\pm$ 0.04	918 $\pm$ 42	

Results and Discussion

Fig. 2 shows specific bindings with ^3H -diazepam 2 nM at 4 portions in the brain of the control and stress groups. In frontal cortex, the stress group indicated a statistically significant increase (37% : $P < 0.005$) of binding as compared with the control. In other regions, no significant changes were observed. Table 1 shows the results of saturation experiments in frontal cortex. K_D showed no difference in either ^3H -diazepam or ^3H -Ro15-1788 bindings, but Bmax indicated in the stress group a statistically significant increase in both ^3H -diazepam binding (33% : $P < 0.025$), and ^3H -Ro15-1788 binding (24% : $P < 0.025$). However K_D and Bmax of ^3H - βCCE binding were not different between control and stress groups.

It may appear extraordinary that receptors in the brain increase in number in such a short period of time as revealed in the present study. However, Paul and Skolnick (1978) observed an increase of Bmax of BDZ-R in rats by inducing convulsive seizure for a few minutes, and Skerritt *et al.* (1981) also reported an increase of GABA binding in mice brain following swimming stress for three minutes. Moreover increases in benzodiazepine binding have been observed following immobilization stress (Braestrup *et al.*, 1979).

It is hardly conceivable that receptors are promptly provided by protein synthesis in stressful conditions. Rapid increase of Bmax shown in this experiment and other similar reports may therefore be considered to be brought about, by swift uncoupling of endogenous ligand and production of free, unoccupied receptors in response to acute stresses.

On the other hand there was no difference in both K_D and Bmax between control and stress group in case of using ^3H - βCCE as a radioligand. $\beta\text{-CCE}$ is basically BDZ agonist, but does not change the action of chloride ion channel. In this reason, $\beta\text{-CCE}$ is called inverse agonist.

Recently Trullas *et al.* (1987) reported the significant role of chloride ion channel in terms of stress response mechanism. It might be considered different attitude between ^3H -diazepam or ^3H -Ro15-1788 and ^3H - β CCE comes from different response of three drugs to chloride ion channel mechanism.

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