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Benzodiazepine effect of ^{125}I-iomazenil–benzodiazepine receptor binding and serum corticosterone level in a rat model

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Abstract

To test the change in free or unoccupied benzodiazepine receptor (BZR) density in response to diazepam, we investigated ^{125}I-iomazenil (^{125}I-IMZ) binding and serum corticosterone levels in a rat model. Wistar male rats, which received psychological stress using a communication box for 5 days, were divided into two groups according to the amount of administered diazepam: no diazepam [D (0)] group and 10 mg/kg per day [D (10)] group of 12 rats each. The standardized uptake value (SUV) of ^{125}I-IMZ of the D (10) group were significantly lower ($P<.05$) than those of the D (0) group in the frontal, parietal and temporal cortices, globus pallidus, hippocampus, amygdala and hypothalamus. The serum corticosterone level ratio in the D (10) group was significantly lower than that in the D (0) group ($P<.05$). From the change in serum corticosterone levels, diazepam attenuated the psychological stress produced by the physical stress to animals in adjacent compartments. From the reduced binding of ^{125}I-IMZ, it is clear that diazepam competed with endogenous ligand for the free BZR sites, and the frontal, parietal and temporal cortices, globus pallidus, hippocampus, amygdala and hypothalamus are important areas in which ^{125}I-IMZ binding is strongly affected by administration of diazepam.

Keywords: ^{125}I-iomazenil; Benzodiazepine receptor; Psychological stress; Diazepam; Communication box

1. Introduction

^{123}I-iomazenil (^{123}I-IMZ) is a radioligand that acts on central benzodiazepine receptors (BZRs) as a partial inverse agonist [1,2]. Central BZRs are located on the alpha subunit of γ-aminobutyric acid ($GABA_A$) receptors, which form chloride ionophores and are believed to be a major inhibitory neuron transmitter receptor in the mammalian brain [3,4]. ^{123}I-iomazenil may be useful for the diagnosis and study of cerebrovascular diseases and neurological and psychological disorders.

We have previously reported that ^{125}I-IMZ–BZR binding is reduced with psychological stress in many areas of the rat brain and is significantly reduced in the cortices, caudate putamen, accumbens nuclei, and amygdala [5]. In addition, we have reported that serum corticosterone levels in rats are elevated when they are exposed to psychological stress [5].

Various hormones are released in response to stress. Activation of the hypothalamic–pituitary–adrenal axis is one of the best-characterized responses to emotive stimuli sufficiently strong to be regarded as stressors [6]. The serum corticosterone level is most frequently used as an index of experimental anxiety [7–9].

Diazepam is a benzodiazepine commonly used as an anxiolytic, anticonvulsant, sedative-hypnotic and muscle relaxant [10–13]. The pharmacological actions of diazepam are due to enhancement of GABAergic inhibition via the $GABA_A$/BZR chloride–channel complex. Although the pharmacological actions and mechanisms of diazepam have been extensively studied, how the pharmacological properties of BZR-specific radiolabeled ligands change with administration of diazepam remains unclear.

In the present study, we investigated changes in ^{125}I-IMZ–BZR binding and serum corticosterone levels with administration of diazepam in rats.

2. Materials and methods

All experiments were done according to the *Guide for the Care and Use of Laboratory Animals* (Institute of Laboratory Animal Resources, 1996).

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Wistar male rats weighing 255–330 g were used. Two or three rats were housed in plastic cages and given food and water ad libitum. The rats were maintained on a 12-h, light–dark cycle (light on from 08:00 to 20:00 h) at a room temperature of 22–25°C and a relative humidity of approximately 45%.

The rats were then divided into two groups according to the amount of administration of diazepam: no diazepam group [D (0)] and 10 mg/kg per day [D (10)] of 12 rats each. All rats received psychological stress using communication box for 5 days. The rats of D (10) were intraperitoneally administered diazepam 20 min before received psychological stress for 5 days.

A communication box was used to establish differences in intraspecies emotional stimuli. This box (32×32×39 cm) was equipped with a floor grid composed of 0.5 cm in diameter stainless steel rods placed 1.3 cm apart. The box consisted of four small compartments (16×16 cm) divided by transparent plastic sheets. Plastic plates were placed on the grid floors of two compartments to prevent the rats from receiving electric shocks (Fig. 1). An electric foot-shock generator (MSG-001, TOYO SANGYOU, Japan) was used to produce a foot shock (3 mA) lasting for 10 s at intervals of 120 s [14,15].

The rats of D (0) and D (10) were individually placed in compartments insulated from the electric grid floor. The boxes were arranged so that the rats in the stress groups were surrounded by those in the compartments in which plastic plates had been placed on the grid floors to prevent them receiving the shocks. These rats did not receive electric foot shocks but were exposed to various emotional stimuli from the rats in the compartments with electric grid floors. These rats were exposed to only psychological stress without physical stress at all. During the stress session, the rats in the compartments with electric grid floors were given intermittent electrical shocks delivered from an electrical shock generator through the grid floor for 1 h. The rats in the compartments with electric grid floors were used only to

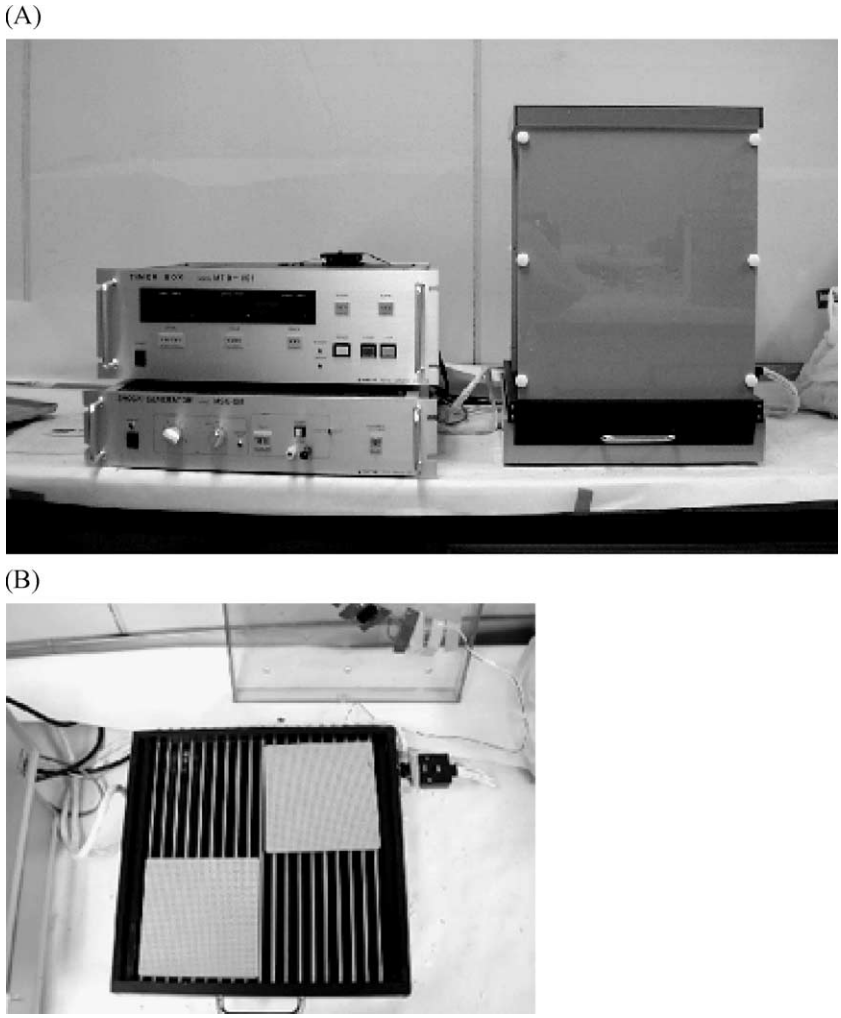

Fig. 1. (A) A communication box system. Upper left: timer box; lower left: shock generator; right: communication box. (B) Inside of a communication box. Rats are placed individually in the foot-shock compartments and non-foot-shock compartments. Foot-shock was delivered through grids of the floor. Plastic plates were placed on the grid floors of two compartments to prevent the rats from receiving electric shocks.