Effect of Benzodiazepines on Synaptosomal Ca^{2+} Transport in Mice with Different Phenotype of Emotional Stress Reactions

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The effects of 5 benzodiazepines on basal and K^+-induced Ca^{2+} concentration in synaptoneurosomes from intact and stressed C57Bl/6 and BALB/c mice were studied *in vitro*. Membrane depolarization induced by low KCl concentrations produced different effects on Ca^{2+} accumulation by synaptoneurosomes from two mouse strains. Benzodiazepines applied *in vitro* exerted no effects on Ca^{2+} influx. In synaptoneurosomes from both C57Bl/6 and BALB/c mice exposed to emotional stress diazepam in a dose of 5 mg/kg reduced the basal and K^+-induced Ca^{2+} accumulation.

Key Words: *calcium; stress; benzodiazepines; Fura-2AM*

C57Bl/6 and BALB/c mice are characterized by different behavioral reactions to emotional stress and different parameters ^{3}H-diazepam binding to cerebral membranes. We previously found that the difference in binding correlates with the difference in cell membrane potential [2]. This prompted us to study more closely the mechanisms regulating membrane-receptor interactions. It is well known that Ca^{2+} plays an important role in the maintenance of cell homeostasis [4,11,12], reaction to emotional stress [4,7,9, 11,12], and the effects of benzodiazepine tranquilizers [8]. This study was aimed at investigation of Ca^{2+} transport in synaptoneurosomes of inbred mice with genetically determined active (C57Bl/6) and passive (BALB/c) behavior in the open field test.

MATERIALS AND METHODS

The experiments were carried out on male C57Bl/6 and BALB/c mice (Stolbovaya Breeding Center) weighing 20-22 g. Before the experiments the animals (10 mice per cage) were kept in separate rooms for at least 2 weeks with 12-h light/dark cycle on a standard diet with free access to water.

Emotional stress was modeled in an open field as described previously [1]. Synaptoneurosomes were isolated from the forebrain [5] and diluted to a protein concentration of 1.2-1.6 mg/ml. Protein concentration was determined as described elsewhere [10].

The preparations were loaded with Fura-2AM [14]. Fluorescence was measured on a Hitachi F-4000 spectrofluorimeter at 340 and 380 nm excitation and 510 nM emission wavelengths. Cuvettes were kept in a thermostat at 37°C. Ca^{2+} concentration was measured as described previously [6].

The data were processed statistically using Student's *t* test.

RESULTS

In synaptoneurosomes from BALB/c mice, changes in Ca^{2+} concentration after incubation with KCl were more pronounced than in synaptoneurosomes from C57Bl/6 mice, which agrees with the previous data on a more labile rearrangement in membranes of BALB/c mice in response to changes in membrane potential [2,13]. Similar regularity was observed in experiments with membranes from mice exposed to emotional stress (Table 1).

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TABLE 1. Diazepam-Induced Changes in Synaptoneurosomal Ca^{2+} Concentration before and after Stress in BALB/c and C57Bl/6 Mice (*M±m*)

Ca^{2+} concentration		Control	Stress	Placebo	Diazepam
Basal	BALB/c (*n*=7-11)	359.7±7.7	337.9±10.9	324.1±4.4*	282.2±3.2*+°
	C57Bl/6 (*n*=8)	331.8±5.9	316.6±5.4	311.9±7.5	298.3±9.3*
K^+-stimulated					
KCl, 5 mM	BALB/c (*n*=3-4)	416.6±29.3	433.5±28.3	393.5±7.7	345.9±7.4+°
	C57Bl/6 (*n*=3)	345.3±4.6	384.6±5.5	329.6±8.3×	315.4±8.7×
KCl, 50 mM	BALB/c (*n*=3-4)	621.1±49.5	581.2±16.8	545.4±6.5	515.7±37.1
	C57Bl/6 (*n*=3-4)	505.4±23.6	551.6±21.3	521.1±16.9	488.4±27.9

Note. Significant difference: *in comparison with the control; +in comparison with stress; °in comparison with placebo; ×in comparison with BALB/c.

Gidazepam (2×10^{-8}-10^{-5}), phenazepam (10^{-7}-10^{-4}), medazepam (5×10^{-9}), cinazepam (5×10^{-5}), and alprozalam (5×10^{-5}) exerted no effects on Ca^{2+} accumulation in synaptoneurosomes from both mouse strains.

In both BALB/c and C57Bl/6 mice, *ex vivo* administration of diazepam (5 mg/kg) reduced the synaptoneurosomal concentration of Ca^{2+} below both the control and poststress values.

These data indicate that changes in the membrane potential have a different effect on membranes of C57Bl/6 and BALB/c mice. More pronounced membrane modifications were noted in synaptoneurosomes from BALB/c mice. This supports the hypothesis that membrane modifications are responsible for reduced sensitivity to benzodiazepines and the development of a fear response in BALB/c mice [3]. It is likely that Ca^{2+} ions play a role in the development of emotional stress reaction in mice of both strains. However, further examinations are needed to explain similar shifts in Ca^{2+} concentration induced by emotional stress and diazepam.

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