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Buspirone attenuates conditioned fear-induced c-Fos expression in the rat hippocampus

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Abstract

The role of hippocampus in the anxiolytic-like effect of buspirone in the conditioned emotional response test (CER, a freezing response), was examined by immunocytochemical detection of the c-Fos protein. It was shown that buspirone at the dose of 0.5 and 1.5 mg/kg i.p. given before test session, which was 24 h after the aversive training, significantly decreased freezing response within a limited dose range of the U-shaped dose–response relationship. Exposure of animals to aversively conditioned context (a contextual fear) induced the production of c-Fos protein in the dentate gyrus, CA-1 and CA-3 layers of the hippocampus. Pretreatment with buspirone (1.5 mg/kg) significantly attenuated the effects of aversive memory on c-Fos protein expression in the CA-1 and CA-3 layers of the hippocampus. These immunocytochemical results support previous data obtained in our laboratory with the help of selective neurotoxic lesions and intrahippocampal drug injections suggesting an important role of hippocampus in the anxiolytic effects of buspirone.

Keywords: Contextual fear; c-Fos protein; Buspirone; Hippocampus; Rats

The 5-HT_{1A} receptor agonists, i.e., derivatives of azapirones, represented by buspirone, are recognized to selectively modulate fear and anxiety in humans. These agonists also represent an alternative to benzodiazepine derivatives for treatment of anxiety disorders. The exact mechanism and site of action of 5-HT_{1A} receptor agonists are not fully known despite years of both preclinical and clinical studies (e.g. [5]). In comparison to benzodiazepines the clinical effect of buspirone is less potent, and evident in some anxiety disorders only. Moreover, the action of buspirone in preclinical models of anxiety is more variable. Previously, we have found that after peripheral drug administration, buspirone showed anxiolytic-like action in a limited dose-range in the Vogel conflict test and in the open field test of neophobia [9,17,18]. Buspirone, at the dose of 2.5 μg/site administered to the dentate gyrus of the hippocampus, increased the time spent on exploration of open arms in the elevated plus maze (EPM), and increased the number of central entries in the open field test [9]. Furthermore, with the help of selective lesions to the serotonergic neurons and intracerebral injections of drugs into the hippocampus, it was shown by us that hippocampal postsynaptic 5-HT_{1A} receptors account for the anxiolytic effects of azapirones in the Vogel conflict and open field tests [17]. Consequently, it has been hypothesized that inhibition of the activity of the hippocampus, secondary to hyperpolarization of neurons after 5-HT_{1A} receptor activation, is an important component of the anti-anxiety action of buspirone and other azapirones [14]. These data are consistent with the other authors' reports showing that suppression of the hippocampal CA1 pyramidal neuronal activity by anxiolytic 5-HT_{1A} agonists in rats is mediated by the postsynaptic 5-HT_{1A} receptors located on pyramidal neurons [19].

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Having in mind all these facts, we have decided to investigate further the role of hippocampus in the anxiolytic-like action of buspirone using an immunocytochemical method. The changes of neuronal activity, accompanying the anxiolytic-like effect of buspirone on the conditioned emotional response (CER, a freezing response), were followed in the rat hippocampus by immunocytochemical detection of the c-Fos protein—the product of expression of the *c-fos* gene, and a marker of changes in neuronal activity. Appropriate control groups of naive, not pretreated with a drug and not conditioned to the experimental procedure animals, along with a group of rats subjected to fear conditioning, but not pretreated with buspirone, were used in parallel to assess a contribution to the *c-fos* gene expression of the accompanying factors, i.e., habituation to the experimental procedure, and memory of an aversive event (a contextual fear).

One hundred and twenty adult, male Wistar rats (220 ± 30 g) bought from a licensed breeder were used in this study. Animals were housed four per cage in standard laboratory conditions under 12-h-light/12-h-dark cycle (lights on at 07:00 h; temperature 20 °C; humidity 70%), with free access to food and water. The study was carried out in accordance with European Communities Council Directive of 24 November 1986 (86/609/EEC). All experiments were approved by the Committee for Animal Care and Use at the Medical University of Warsaw. All experiments were conducted during light period of the day–night cycle. Each experimental group consisted of seven to eight animals.

To reduce non-specific c-Fos expression caused by injection stress, all animals were habituated and injected with saline for 4 days before experiment. Each animal received seven injections in total (four before and three during the conditioned fear test). On the first experimental day, animals were divided into following groups: C1, group given repeated saline injections in the home cage to establish the basal level of c-Fos expression (seven injections, the last injections 2.5 h before decapitation); C2, saline administered animals subsequently exposed to the conditioning cage for 10 min, 2 h before decapitation (seven injections, the last injections 2.5 h before decapitation). This group was designed to control for the effects of novelty; E1, animals subjected to the full conditioning procedure, and given the last injection of saline 30 min before exposure to the conditioning box and 2.5 h before decapitation (seven saline injections). This group was designed to study the effect of a conditioned fear on c-Fos expression; E2, animals subjected to the full conditioning procedure (six saline injections), and administered buspirone (1.5 mg/kg) 30 min before exposure to the conditioning box (the seventh injection) and 2.5 h before decapitation. This group was designed to study the influence of buspirone on conditioned fear-induced c-Fos expression. During the contextual fear conditioning procedure, the animals received injections 30 min prior to the start of behavioral testing.

Buspirone (Anpharm, Poland) was dissolved in 0.9% NaCl and administered intraperitoneally (2 ml/kg). The following doses of buspirone were used: 0.5, 1.5 and 3.0 mg/kg, on the third, final day of the experimental procedure, 30 min before the test in a random order. Based on these results the dose 1.5 mg/kg was chosen for the immunocytochemical part of the study.

The experiment was performed using a computerized fear-conditionining system (TSE, Bad Homburg, Germany), during three consecutive days (habituation-, training-, and test day), in the same testing box (36 cm × 21 cm × 20 cm) under constant, white noise (65 dB). The box was cleaned after each trial with 95% ethanol. On the first day, animals were placed separately for 2 min in the box for adaptation to experimental condition. The following day, a training day, after a 5-min pause, the animals received three 1-s footshocks repeated every 59 s (a train of 0.7 mA, 150-ms long impulses repeated every 300 ms), and then remained in the box for additional 3 min after the last footshock was delivered. On the third experimental day animals received either drug or saline 30 min before testing, and then the freezing behavior of the animals was analyzed for 10 min in the same box. The freezing was defined as absence of any movements except for those required for respiration [11].

After decapitation (2.0 h after onset of testing of freezing behavior on the third experimental day, and 2.5 h after saline or buspirone injection), the brains were removed and stored in −70 °C. The immunocytochemical reaction was performed on slide-mounted brain sections according to the procedure described below. Coronal 15 μm cryostat sections from each animal were cut and mounted on silan-coated slides, and fixed in 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4) for 15 min. The specimens were then washed twice (2× 15 min) in 0.01 M PBS solution (pH 7.4), incubated in 3% H_2O_2 solution for 30 min to block the activity of endogenous peroxidase, then washed again in 0.01 M PBS solution (pH 7.4) twice (2× 15 min), and incubated in a 3% normal goat serum (NGS) blocking solution. Subsequently, slide-mounted brain sections were incubated in rabbit polyclonal c-Fos IgG diluted at 1:20000 (sc-52x, Santa Cruz, USA) in temperature 4–8 °C for 72 h, washed in 0.01 M PBS solution (pH 7.4) three times (3× 15 min), then incubated with biotynylated anti-rabbit IgGs (Vector Laboratories, CA, USA) for 2 h, rinsed in 0.01 M PBS solution (pH 7.4) twice (2× 15 min), and incubated with avidine–biotine–peroxydase complex (Vector Laboratories) for 1 h. Finally, after being washed in 0.01 M PBS solution (pH 7.4) twice, slide-mounted brain sections were immunoreacted with a solution containing Tris, 0.03% diaminobenzidine hydrochloride (DAB) and 0.003% H_2O_2. The slides were then dehydrated in alcohol, dewaxed and cleared in xylene, and coverslipped with the use of the histological mountant [20]. Fos-like immunoreactivity was assessed by light microscopy (Olympus BX-51 light microscope, Camedia Master C-3040 digital camera) at a magnification of 40×. The number of c-Fos-positive nuclei was counted bilaterally with the use of a computerized image analysis system (Olympus DP-Soft version 3.2 software) in the following subregions of hippocampus: CA1, CA3, DG, and represented also as a whole hippocampus.