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Research report

β-Adrenoceptor blockade depresses molecular and functional plasticities in the rat neocortex

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ABSTRACT

β-Adrenergic receptor stimulation can significantly facilitate synaptic potentiation in the hippocampus and enhance memory processes, but its effect on neocortical plastic mechanisms is less conclusive. In the present study we determined the effect of propranolol, a β-adrenoceptor antagonist, on long-term potentiation (LTP) induced *in vivo* in rat occipital cortex by tetanizing stimulation of corpus callosum and observed a dose-dependent inhibition of LTP. We further administered propranolol through mini-osmotic pumps during 3 days, and observed the performance of rats in a complex operant conditioning learning paradigm and assessed the expression of brain-derived neurotrophic factor (BDNF) in the occipital cortex. Propranolol exposure depressed both the number of reinforced responses in the operant conditioning task and BDNF expression in occipital cortex. Taken together, our results suggest that propranolol impairs memory formation by inhibiting cortical LTP induction and associated BDNF expression.

1. Introduction

Noradrenaline critically influences learning and memory by modifying hippocampal [2] and neocortical functions [2,21]. Long-term potentiation (LTP) is widely accepted as the best-studied model for neurophysiological mechanisms underlying learning and memory formation. While numerous studies indicate that β-adrenergic receptor stimulation can significantly facilitate hippocampal LTP [23,26], the effects of β-adrenoceptor agonists and/or antagonists on neocortical LTP are less conclusive. For instance, Brochër et al. [4] reported that β-adrenoceptor agonists raised the probability that tetanic stimuli induced LTP in the rat visual cortex, while Nowicky et al. [18] argued that noradrenaline and isoproterenol had no effect on the depolarizing slope or peak amplitude of sub-threshold excitatory postsynaptic potentials, meaning that drug application did not increase the probability of induction of LTP.

Differential loss of extrinsic noradrenergic activity in the slice preparations used in the above mentioned studies might account in part for these inconsistencies. Thus, we studied the effect of the β-adrenoceptor antagonist propranolol on LTP induced *in vivo* by tetanizing stimulation applied to the occipital cortex in anesthetized rats. Besides, on the basis that activity-dependent transcription of brain-derived neurotrophic factor (BDNF) is required for network-level adaptations in cortical circuits, such as those critically involved in translating LTP associated to sensory experience into learning and memory [10], the effect of long-lasting propranolol administration upon occipital cortex BDNF expression and on a complex operant conditioning in Skinner box was also studied. As a whole, the present results showed that propranolol administration leads to a dose-dependent reduction of occipital cortex LTP, altogether with depressing the number of reinforced responses in the operant conditioning task and the BDNF expression in the occipital cortex.

2. Materials and methods

This investigation was performed following protocols approved by the Committee for the Ethical Use of Experimental Animals at INTA-University of Chile, in accordance to the NIH Guide for the Care and Use of Laboratory Animals [16].

2.1. Electrophysiological studies

Electrophysiological experiments were carried out in 28 anesthetized (1.5 g/kg i.p. urethane), artificially ventilated, male Sprague–Dawley rats weighing 200–250 g as described elsewhere [14,24]. Electrical stimulation of the corpus callosum (CC)

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was carried out by means of a bipolar electrode (two braided 100-μm-diameter insulated wires with a 0.5-mm tip separation) that penetrates the right occipital cortex at de Groot's coordinates $A = 0.0$ mm, $L = 3.5$ mm, according to the atlas of Pellegrino and Cushman [19]. Cortical-evoked responses were recorded from the left occipital cortex with a surface monopolar silver ball electrode of 0.5-mm diameter located at similar coordinates to those utilized for transcortical stimulation of the CC. Test stimuli consisted in 100-μs duration square-wave pulses generated at 0.1 Hz by means of a Grass S11 stimulator in conjunction with a Grass SIU-5 stimulus isolation unit and a Grass CCU 1A constant current unit (all Grass equipments from Astro-Med Inc., West Warwick, RI, USA). Before the beginning of each experiment, a full input–output series was performed at a stimulus intensity of 300–1100 μA, and test stimuli with stimulation intensity yielding responses with peak-to-peak amplitude of 50% of maximum were used for the remainder of the experiment. After a 30-min stabilization period, a 5-min control period of basal responses (30 averaged responses) was recorded; then rats received a single dose of DL-propranolol (1.25, 2.5, or 5 mg/kg, i.p.; $n = 7$ for each group). Seven rats receiving i.p. saline served as controls. DL-propranolol hydrochloride (from Sigma–Aldrich, St. Louis, MO, USA) or saline were administered in the same volume (100 μl/100 g body weight). Fifteen minutes after drug or saline administration, a new 5-min control period of responses was recorded. Afterward, a tetanizing stimulus was applied, consisting in a single train of 100-μs duration square-wave pulses at 312 Hz and 500-ms duration, with intensity 50% higher than test stimuli. Recordings were amplified using a 0.8–1000 Hz bandwidth (Grass P-511 preamplifier, Astro-Med Inc., West Warwick, RI, USA), displayed on a dual trace digital oscilloscope (Philips PM 3365A, Amsterdam, The Netherland), digitized at the rate of 10 000/s by an A/D converter interfaced to a microcomputer (Pentium PC, Acer Inc., Taipei, Taiwan) and stored in hard disk for retrieval and off-line analysis. In all experiments, body temperature and expired CO_2 were monitored and remained within normal limits. The efficacy of the tetanizing train to potentiate cortical-evoked responses was evaluated by measuring the peak-to-peak amplitudes [14,24]. At the end of the electrophysiological experiments, the animals were euthanized by an overdose of sodium pentobarbital.

2.2. Operant conditioning task

Behavioral assessment of operant conditioning was performed in a Skinner box (from Laffayette Instrument Co., Laffayette, IN, USA), employing 12 male Sprague–Dawley rats receiving subcutaneous chronic administration of DL-propranolol (3 mg/kg/day) or saline during 3 days by means of an Alzet 2ML1 mini-osmotic pump (Direct Corporation, Cupertino, CA, USA) delivering 10 μl/h. Rats were anesthetized with 300 mg/kg i.p. of chloral hydrate (from Sigma–Aldrich, St. Louis, MO, USA) and a mini-osmotic pump was implanted subcutaneously on the back of each animal and the skin was sutured. To avoid new anesthesia and surgery, the pump was not removed from the experimental animals, meaning that behavioral assessment occurred under continuous propranolol administration. Within the following 3 days prior to testing, the rats were submitted to a food restriction schedule, where animals were given only a few pellets which were subsequently used as unconditioned reinforcers in the operant conditioning task. The testing apparatus was a metal Skinner box except for the front wall which was made of opaque plexiglass, containing an omni-directional vertical metal lever, a luminous stimulus, a horizontal metal press bar and a food dispenser. The Skinner box was commanded by a Dell computer provided with an animal behavioral environment test (ABET) software package (from Laffayette Instrument Co., Laffayette, IN, USA), so that, after a lever-press, the light turned on and the rat had to press the bar within the following 5 s to get pellet from the dispenser. This was considered as a successful reinforced response. On the third day after mini-pump implantation, rats receiving saline were first submitted, individually, to four modeling sessions of 10 min each one with 1-h inter-session interval. Modeling consisted in training rats to get the three-link concurrent-chain schedule of reinforcement (omni-directional lever displacement – light turning on – bar pressing – pellet dispensing). In this passive stage the experimenter turns the light on (conditioned reinforce) when the rat approximates and gently touches the lever without moving it, and the experimenter dispenses food (unconditioned reinforce) to the rat when it gently touches the bar without pressing it. Thus, pellet is dispensed only when the light is on. For propranolol-treated rats, the number of modeling sessions (10 min each one with 1-h inter-session interval) was incremented until the rats reached a similar reinforcement than control saline rats. The number of bar touches, which is similar to the number of pellets dispensed, was measured as significant parameter for evaluating acquisition of the operant conditioning task. One hour after finishing the modeling stage, rats were individually submitted to five testing sessions of 10 min each with 1-h inter-session interval. Testing sessions differed from modeling in that pellet is dispensed by the computer software whenever the rat presses successively the lever (light on) and the bar in less than 5 s, which is scored as a successful response. The mean of successful reinforced responses in each testing session was measured as significant parameters for evaluating operant conditioning performance learned during modeling sessions.

2.3. BDNF determinations

BDNF protein concentration was measured in 10 additional male Sprague–Dawley rats receiving subcutaneous chronic administration of DL-propranolol (3 mg/kg/day) or saline during 3 days by means of an Alzet 2ML1 mini-osmotic pump delivering 10 μl/h, as indicated above. At day 3 after pump implantation the animals were euthanized by decapitation, the brain removed, and the left and right occipital cortices were dissected out. Samples were weighed and stored at −80 °C before use. Afterwards, the tissues were examined for expression of BDNF protein level by ELISA, as described elsewhere [9].

Whole samples of occipital cortex were homogenized in ice-cold lysis buffer, containing 137 mM NaCl, 20 mM Tris–HCl pH 8.0, 1% Triton X-100, 10% glycerol, and 2 μl/ml protease inhibitor cocktail P8340 (Sigma–Aldrich, St. Louis, MO, USA). The tissue homogenate solutions were centrifuged at 14 000 × g for 5 min at 4 °C, the supernatants collected, diluted in buffer DPBS, acidified in 1 N HCl, then incubated at room temperature and neutralized with 1 N NaOH solution. BDNF levels were assessed using the E-Max ImmunoAssay system ELISA kit (Promega, Co., Madison, WI, USA). Briefly, standard ELISA plates were incubated overnight at 4 °C with a monoclonal anti-BDNF antibody. The plates were incubated in 1x blocking solution and sample buffer. Serial dilutions of known amounts of BDNF ranging from 0 to 500 pg/ml were used in duplicate for standard curve determination. BDNF standards and supernatants of brain tissue homogenates were incubated with a secondary anti-human BDNF polyclonal antibody, as specified by manufacturers. A species-specific antibody conjugated to horseradish peroxidase was used for tertiary reaction, and TMB one solution was used to develop color in the wells. This reaction was terminated with 1 N hydrochloric acid, and absorbance was recorded at 450 nm in a microplate reader within 40 min. The neurotrophin values were determined by comparison with the regression line for BDNF and expressed as pg BDNF/mg wet weight. Using this kit, BDNF can be quantified in the range of 7.8–500 pg/ml.

2.4. Statistical analyses

Data are reported as means ± S.E.M. All statistical analyses were performed with GraphPad Prism version 3.00 (GraphPad Software, Inc., San Diego, CA, USA). Differences in time-course of cortical LTP after each dose of propranolol (intergroup comparisons) were determined using repeated measures ANOVA followed by Bonferroni *post hoc* test. The area under the curves obtained in drug- or saline-treated animals was measured using the Microcal Origin 6.0 software (Microcal Software, Northampton, MA, USA), and intergroup comparisons were performed using one-way ANOVA followed by Bonferroni *post hoc* test. Propranolol-induced changes (in percentage) of the area under the curves (relative to the saline group) were plotted against log dose of propranolol, and the ED_{50} was calculated from linear fitting by utilizing standard interpolation procedures (Origin 6.0, Microcal Software). Behavioral results were expressed as the number of bar touches during four–five consecutive sessions of modeling as well as the number of successful reinforced responses after five consecutive testing sessions, observed in saline- and propranolol-treated rats, and intergroup comparisons were performed using repeated measures ANOVA followed by Bonferroni *post hoc* test. For analyzing results of BDNF protein expression, intergroup comparison between propranolol and saline groups was made using unpaired Student's *t*-test.

3. Results

3.1. Effect of propranolol on occipital cortex LTP

Fig. 1 A shows a scheme indicating the position of the stimulating and recording electrodes in the rat brain, as well as the averaged occipital cortical response evoked by contralateral stimulation of the corpus callosum in a control rat, prior and after the application of a tetanizing train. Fig. 1B shows that field cortical responses evoked in saline-treated animals exhibited about 60% increase in peak-to-peak amplitude after application of the tetanizing stimulus, an effect that was maintained throughout the recording period. Fig. 1B also shows that 5 and 2.5 mg/kg i.p. propranolol did not change the peak-to-peak amplitude of cortically evoked responses by its self, but completely prevented the induction of LTP by the tetanizing stimulation, as compared to saline injected controls. 1.25 mg/kg propranolol i.p. did not produce any effect. Fig. 1C shows areas under curves shown in Fig. 1B, revealing a dose-dependent relationship between propranolol dosage and LTP reduction. Linear regression analysis (Fig. 1D) allowed to calculate $ED_{50} = 1.67$ mg/kg for i.p. propranolol. The 95% confidence interval range is 1.35–2.22 mg/kg i.p.

3.2. Effect of propranolol in the three-link concurrent-chain schedule of reinforcement

Fig. 2A shows that propranolol-treated rats performed similarly to saline controls during the first three sessions of modeling, while