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Neuroscience Letters 402 (2006) 253–258
Neuroscience Letters
www.elsevier.com/locate/neulet

Yohimbine acts as a putative in vivo $\alpha_{2A/D}$-antagonist in the rat prefrontal cortex

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Received 21 November 2005; received in revised form 27 March 2006; accepted 10 April 2006

Abstract

Yohimbine has been widely used as α_2-adrenergic receptor antagonist in neurophysiological research and in clinical therapy. In this study, we provide in vivo electrophysiological evidence, that microiontophoretic application of yohimbine (YOH) inhibits spontaneous activity of prefrontal neurons of the rat. By microiontophoretic application of the α_{2A}-receptor antagonist BRL44408 (BRL), the effects of YOH could be mimicked, indicating that the action of YOH is manifested through $\alpha_{2A/D}$-receptor mechanisms. Furthermore, the inhibiting effects of YOH or BRL were blocked by α_{2B}-receptor antagonist imiloxan. In concert with previous microiontophoretic data, the present findings suggest that α_2-receptor antagonist YOH predominantly acts on the $\alpha_{2A/D}$-receptor subtype in vivo. Furthermore, we hypothesize that this action is manifested via deactivation of autoreceptors causing increased norepinephrine release, finally inhibiting postsynaptic neurons through the activation of α_{2B}-receptors.

Keywords: Alpha-2 adrenergic receptors; Microiontophoresis; Norepinephrine; Prefrontal cortex; Yohimbine

Yohimbine (YOH) is a widely applied substance previously described as a non-selective α_2-adrenoceptor antagonist in the nervous system. It has significant antinociceptive action [8], and it has been shown to be effective in the treatment of obesity [19] and psychiatric disorders, such as depression or narcolepsy [28]. Yohimbine has been used in neuropharmacological research of stress [21], anxiety [7] and memory processes [3]. In rodent experimental models, these functions have been suggested to be highly dependent on norepinephrinergic (NEergic) mechanisms intrinsic to the medial prefrontal cortex (mPFC) [12,23,27].

Intracerebral α_2-adrenoceptors have been divided into four subtypes: α_{2A}, α_{2B}, α_{2C} and α_{2D} [4]. The human α_{2A}-subtype is homologous with the rodent α_{2D}-subtype [16] often referred as $\alpha_{2A/D}$-subtype [29]. Although $\alpha_{2A/D}$-subtype also acts presynaptically as autoreceptor [29], the majority of $\alpha_{2A/D}$-receptors in the brain are postsynaptic [1]. The $\alpha_{2A/D}$-autoreceptors inhibit release of norepinephrine (NE) into the synaptic cleft, as their stimulation decreases the extracellular levels of NE [5,13,31]. In addition to $\alpha_{2A/D}$-receptors, on the postsynaptic side, β_1-, α_1-, α_{2B}- and α_{2C}-receptors are also expressed [6,16]. While β_1- and α_1-receptors depolarise both subcortical [2,6] and cortical neurons [17,20], through the activation of intracellular cAMP- and inositol 1,4,5-trisphosphate (IP_3) second messenger systems, only $\alpha_{2A/D}$, α_{2B}- and α_{2C}-receptors are known to hyperpolarize neurons by the inhibition of cAMP synthesis [2,6].

There are three major conflicting interpretations of the action mechanisms of YOH in the brain. Firstly, in most cases, YOH has been shown as a general α_2-adrenoceptor antagonist [3,10]. Secondly, YOH reportedly acts as a subtype-specific antagonist on α_{2B}/α_{2C} [16,30] or $\alpha_{2A/D}$ receptors [11,32] in vitro. Thirdly, YOH has been used as a presynaptic α_2-receptor antagonist without considering a confounding effect through postsynaptic α_2 receptor mechanisms [9,24]. If YOH mainly antagonizes the presynaptic autoreceptors, it should increase the release of NE as it was formerly described [5,24,28,31,32].

Previous data from our laboratory [15] and other independent studies [26] demonstrated that YOH decreases maintained firing rate in the rat and the monkey PFC, respectively. Yohimbine inhibition was successfully mimicked with α_2-agonist clonidine (CLON), but not by α_1-antagonist prazosin, suggesting a primary action of YOH on α_2 receptors.

Although, in vitro data is available suggesting the $\alpha_{2A/D}$ receptor-specific action of YOH [11,32], there is no in vivo electrophysiological evidence for similar action of YOH. Because of the widespread pharmacological [8,19,28] and behavioural

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doi:10.1016/j.neulet.2006.04.011

[3,7,21] application of YOH, it is important to define its in vivo action accurately. In this study, we aimed to determine the in vivo pharmacological action of YOH on the maintained firing activity of medial PFC (mPFC) neurons in the rat. The action of YOH was confirmed by means of combined extracellular single neuron recording and microiontophoresis of YOH and various other α_2-adrenergic receptor agents.

Experiments were approved by the Animal Care Committee at our Institution (University of Pécs, Hungary), in compliance of international standards (NIH Guidelines) for the care and use of laboratory animals. Twenty male Long Evans rats (Charles River Laboratories, Gödöllő, Hungary) were used during experiments. Anaesthesia was induced with a single injection of ketamine (100 mg/kg, SBH, Hungary) and maintained with 20% of the initial dose administered in approximately every 45 min thereafter for a maximum of three times. Stereotaxic coordinates for the targeted regions of the mPFC according to Paxinos and Watson [22] were: AP (from bregma) 2.7–3.7 mm; L 0.2–1.0 mm; V (from the dura) 1.0–5.0 mm.

Seven-barrelled micropipettes were used for recording and microiontophoresis (Carbostar-7, Kation Scientific, USA). The impedance of the central recording channel was 0.2–0.6 MΩ (at 50 Hz), and the impedance for each drug channel was 10–50 MΩ. One of the drug-channels (filled with 0.5 M NaCl or pontamine sky blue or methylene blue solution) was used for the application of a continuous balancing current, while four other capillaries were filled with the following substances: α_2-antagonist YOH (ICN, 10 mM, dissolved in 30% DMSO/70% distilled water), subtype selective $\alpha_{2A/D}$-antagonist BRL44408 (BRL) (Tocris 10 mM, dissolved in distilled water), subtype selective α_{2B}-antagonist imiloxan (IMI) (Tocris, 10–80 mM dissolved in distilled water) and subtype specific $\alpha_{2A/D}$-agonist guanfacine (GUA) (Tocris, 10–80 mM, dissolved in distilled water). The remaining electrode channel was filled with GABA (Sigma, 50 mM, dissolved in distilled water) or kainic acid (Sigma, 50 mM, dissolved in distilled water) for inhibitory or excitatory control purposes, respectively. All compounds, except kainic acid, were ejected as cations by individual constant current circuits (Neurophore BH2, Medical Systems Corp., USA). To determine the overall electric charge (nC) passed through the electrode tip during drug applications, we multiplied the applied electric current (nA) by the duration of pump opening (s).

Extracellular single unit activity was recorded and passed through an analogue-digital converter interface (Power 1401, CED, Cambridge, UK) to an IBM-compatible microcomputer. Spike sorting and data analysis was performed by Spike2 software (CED, Cambridge, UK) to ensure that data were always recorded from single neurons. Frequency histograms of neuronal discharge activity were computed and displayed in cycles per seconds (cps, Hz). Baseline activity was recorded for 60 s before starting a drug application trial. A neuron was considered responsive to a treatment when its firing rate changed ±20% respective to its baseline level. Responses were assessed during microiontophoresis and, typically, for 60 s after the termination of drug ejection. In the case of long lasting drug application trials (>100 s), neuronal activity was processed for a minimum of 60 s, or until the activity of the cell first reached its pre-trial firing rate. Neuronal activity was converted to normalized values (Table 1, Fig. 2B) representing the percentage of activity changing between the neuron's baseline firing rate and its response to treatment (±standard deviation, S.D.). Statistical comparison between pre- and post treatment firing activity was performed using Student's paired *t*-tests. Neuronal responses were then analysed by one-way ANOVA between three response categories (excitation, inhibition, no response). The threshold for significance for all statistical comparisons was set to $p < 0.01$.

Recorded areas were microiontophoretically labelled with pontamine sky blue or methylene blue through continuous application from the current balancing channel of the electrode during the recording sessions, as described elsewhere [14]. At the end of each recording session, animals were perfused transcardially with saline solution followed by 4% paraformaldehyde. Brains were postfixed and rinsed in PBS. Native 40 μm slices were made and sections were studied under a light microscope.

We recorded extracellular single unit activity of 119 mPFC neurons in conjunction with 889 different drug application trials. The mean baseline firing activity of the neuronal pool was 12.37 ± 3.24 Hz. Localization of each electrode track was successful, but histological analysis for the localization of individual neurons was possible only in 90% (107/119) of recorded positions. If a neuron fell outside the target area, or its position could not be located within the electrode track, it was excluded from further analyses. The distribution of localized recorded neurons among the target subregions of the mPFC were the following: anterior cingulate: 27, prelimbic: 49, infralimbic: 31. As neuronal responsiveness did not reveal subregional differences, the target area will be referred as mPFC throughout the text.

Yohimbine: The effect of YOH microiontophoresis was inhibitory in 55 of 63 tested mPFC neurons (87%) to 0.51 ± 0.18 of the pre-treatment spontaneous activity (one-way ANOVA, $F(1,191) = 48.85$, $p < 0.0001$) (Fig. 1A, B and D). Firing rate suppression typically lasted 100–1500 s and was proportional to the applied electric charge.

BRL 44408: The subtype selective $\alpha_{2A/D}$-adrenoceptor antagonist BRL induced similar electrophysiological effects to YOH (Fig. 1A–D). Similarly to YOH, BRL also decreased the spontaneous firing rate (0.49 ± 0.17) in 68 of 82 neurons (83%) (one-way ANOVA, $F(2,320) = 282.54$, $p < 0.0001$). BRL-induced suppression was recorded with all applied currents (10–70 nA, 100–500 s), while excitations were only recorded in 4% of tested neurons with lower ejection current (10–20 nA) (Table 1). Overall, the application of BRL induced shorter inhibitory responses of maintained firing activity than that of YOH (mean duration of inhibition (±S.E.M.) YOH: 504.48 ± 67.46, BRL: 104.58 ± 14.64, Student's *t*-test, $p < 0.0001$). Fig. 2A depicts the firing rate suppression as a function of the duration of microiontophoretic YOH or BRL application.

Imiloxan: The majority of tested neurons (47/73, 64%) did not change their activity during IMI ejection, even when IMI was applied with several different protocols from short to long duration (Fig. 1B, C, D and F). However, 28% of IMI applications resulted in excitation of the recorded neurons (Fig. 1E), and only a small portion of neurons (6/73, 8%) were suppressed by IMI (one-way ANOVA, $F(2,161) = 64.82$, $p < 0.0001$) (Table 1).