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Research paper

Yohimbine is a 5-HT_{1A} agonist in rats in doses exceeding 1 mg/kg.

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HIGHLIGHTS

- Alpha2-adrenoblocking properties of yohimbine are observed in doses below 1 mg/kg.
- In doses exceeding 1 mg/kg yohimbine is a 5-HT_{1A}-agonist.
- Yohimbine reinstates drug-seeking behavior or antagonizes general anesthesia in high doses (more than 1 mg/kg).

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ABSTRACT

Yohimbine is a prototypical alpha2-adrenergic receptor antagonist. Due to its relatively high selectivity, yohimbine is often used in experiments whose purpose is to examine the role of these receptors. For example, yohimbine has been employed at doses of 1–5 mg/kg to reinstate drug-seeking behavior after extinction or to antagonize general anesthesia, an effects presumably being a consequence of blocking alpha2-adrenergic receptors. In this report we characterized dose-dependent autonomic and behavioral effects of yohimbine and its interaction with an antagonist of 5-HT_{1A} receptors, WAY 100,635. In low doses (0.5–2 mg/kg i.p.) yohimbine induced locomotor activation which was accompanied by a tachycardia and mild hypertension. Increasing the dose to 3–4.5 mg/kg reversed the hypertension and locomotor activation and induced profound hypothermia. The hypothermia as well as the suppression of the locomotion and the hypertension could be reversed by the blockade of 5-HT_{1A} receptors with WAY 100635. Our data confirm that yohimbine possesses 5-HT_{1A} properties, and demonstrated that in doses above 1 mg/kg significantly activate these receptors.

1. Introduction

Yohimbine is a prototypical alpha-2 adrenoreceptor antagonist in neuropharmacological studies [16]. It was and still is widely used in various experimental studies *in vitro* [10,19,34] and *in vivo* in conscious animals [3,5,7] and as an antagonist of general anesthesia [11,22,24,48].

Importantly, yohimbine has also been reported to evoke responses through dopaminergic [40], alpha1-adrenergic [13,16], 5-HT_{1A} [50,51], and benzodiazepine [29] receptors. The ability of yohimbine to act as a partial agonist for the human 5-HT1A receptor was demonstrated using receptors expressed in cell lines [1]. Hypothermia, induced by yohimbine in rats [21,32] was linked to the activation of 5-HT_{1A} receptors [32].

A major limitation of the above-referenced studies is that they do not provide data establishing the relative receptor selectivity of the doses of yohimbine employed in conscious animals. If yohimbine evokes some action in doses which are non-specific for alpha2-adrenoreceptors, then its pharmacological action needs to be interpreted with caution. For example, yohimbine–induced reinstatement of drug-seeking behavior is usually assumed to be alpha2-adrenoreceptor-mediated based on the widely known alpha2-blocking properties of the drug [2,4]. However, alpha2-receptor antagonist RS-79948 did not trigger reinstatement despite it blocked effects of clonidine [46]. Also, in many studies yohimbine was used in high doses (1–5 mg/kg), which exceed those sufficient to block alpha2-adrenoreceptors.

To determine the doses of yohimbine which significantly activate 5-HT_{1A} receptors in conscious rats we studied dose-dependence of the effects of yohimbine and identified those mediated by 5-HT_{1A} receptors by using WAY 100,635, a specific antagonist of these receptors.

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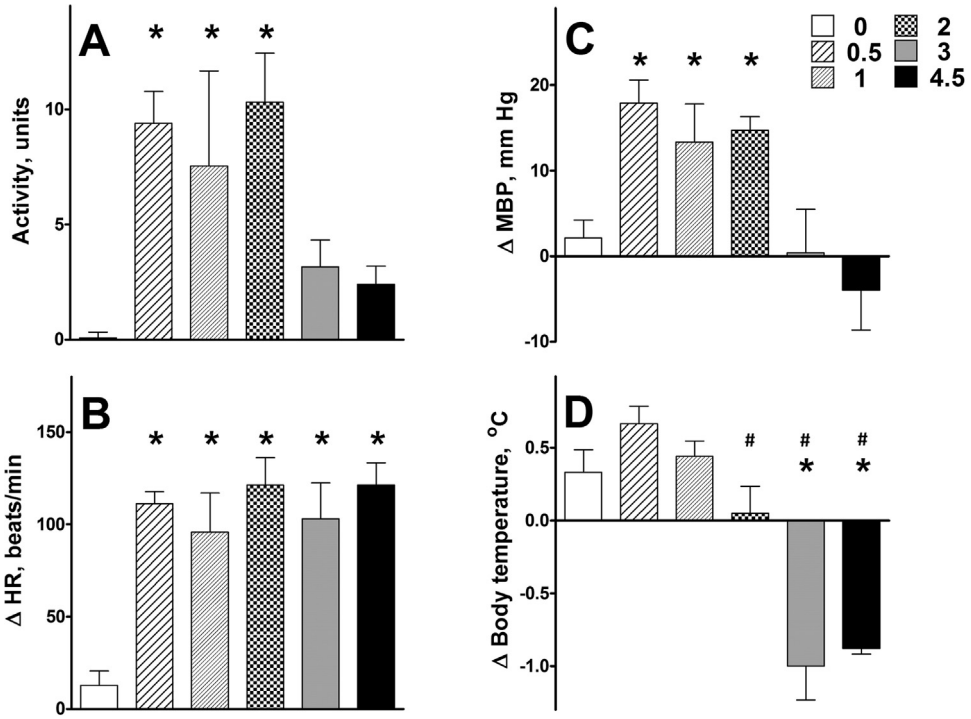

Fig. 1. Physiological responses to intraperitoneal injection of saline or various doses of yohimbine. The data are averages of locomotor activity (A), heart rate (B), mean blood pressure (C) and body temperature (D) over interval of 15–30 min after injection. * – significant difference from saline ($p < 0.05$). # – significant difference from the lowest dose (0.5 mg/kg, $p < 0.05$).

2. Materials and methods

2.1. Animal model

Male Sprague-Dawley rats (250–300 g) were used for all experiments. The animals were individually housed under standard controlled conditions (lights on 07:00–19:00, room temperature of 23–25 °C) with free access to food and water. All procedures described were approved by the IACUC of the Indiana University School of Medicine and followed NIH guidelines.

Rats were implanted with telemetric transmitters (PXT, Transoma Med, St.Paul, MN) under isoflurane anesthesia as previously described [47]. After at least seven days of recovery, rats were brought to experimental room, placed on receivers of telemetric data acquisition system (LabPro 3.11, Data Sciences Int., St.Paul, MN) and allowed to adapt to experimental conditions. All animals for which data are reported remained in good health throughout the course of surgical procedures and experimental protocols as assessed by appearance, behavior, and maintenance of body weight.

2.2. Drugs

Yohimbine hydrochloride and WAY100635 (WAY) were obtained from the Sigma–Aldrich (St. Louis, MO). WAY was dissolved in sterile saline. Yohimbine was first dissolved in an aliquot of distilled water under sonication, and then an equal volume of hypertonic saline (1.8% solution of NaCl in distilled water) was added.

2.3. Experimental protocols

All injections were performed between 11:00 am and 2:00 pm to avoid the effect of circadian variability. Two experimental series were performed.

In the first series of experiments, thermal, locomotor, and cardiovascular responses to various doses of yohimbine were studied. Five doses of yohimbine (0.5, 1, 2, 3, or 4.5 mg/kg in a volume of 1 ml/kg) or sterile saline were given i.p. Animals ($N = 4$) received all doses of yohimbine in random order allowing two days between experiments.

In second series of experiments, three groups of rats ($N = 5$ each) were prepared. Each rat was given two identical i.p. injections of either 0.5 or 3 mg/kg of yohimbine or vehicle separated by 2 days. Administration of yohimbine or saline was preceded by i.p. injection of either WAY (0.5 mg/kg in 1 ml/kg of saline) or saline. The selection of pretreatment for first trial was done by randomization. If in first trial the pretreatment was WAY, than pretreatment for the second trial was saline and vice versa.

2.4. Statistical analysis

The results are presented as the mean ± SEM. For bar graphs and statistical comparisons we have averaged parameters between 15 and 30 min after injection of yohimbine, because this interval is close to maximal changes after both 0.5 and 3 mg/kg yohimbine. Baseline levels of activity, body temperature, heart rate (HR), and mean blood pressure (MBP) did not differ between groups across the series of experiments, so changes from baseline were analyzed unless specially noted.

Results were compared using a one way (series 1) or two-way (series 2) ANOVA with repeated measures followed by a Duncan post hoc test, where appropriate. A value of $p < 0.05$ was considered to indicate a significant difference.

3. Results

Yohimbine dose-dependently affected all of the studied parameters: heart rate, blood pressure, body temperature, and locomotion (Fig. 1, locomotion $F(5,18) = 4.1$; $p = 0.01$; HR $F(5,18) = 7.8$;