



Research paper

Yohimbine is a 5-HT_{1A} agonist in rats in doses exceeding 1 mg/kg.Dmitry V. Zaretsky^{a,*}, Maria V. Zaretskaia^a, Joseph A. DiMicco^b, Daniel E. Rusyniak^{a,b}^a Department of Emergency Medicine, Indiana University School of Medicine, Indianapolis, IN 46202 USA^b Department of Pharmacology and Toxicology, Indiana University School of Medicine, Indianapolis, IN 46202, USA

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.

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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-HT_{1A} 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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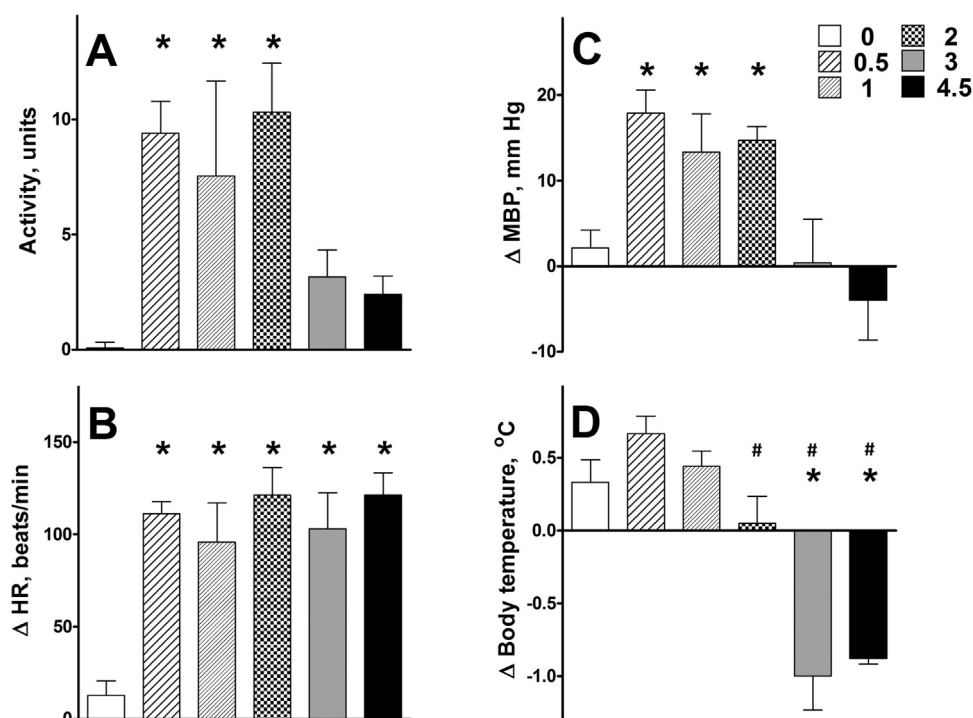


Fig. 1. Physiological responses to intraperitoneal injection of saline or various doses of yohimbine. The data are averages of locomotor activity (A), heart rate (B), mean blood pressure (C) and body temperature (D) over interval of 15–30 min after injection. * – significant difference from saline ($p < 0.05$). # – 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 \pm 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 $F(5,18) = 7.8$;

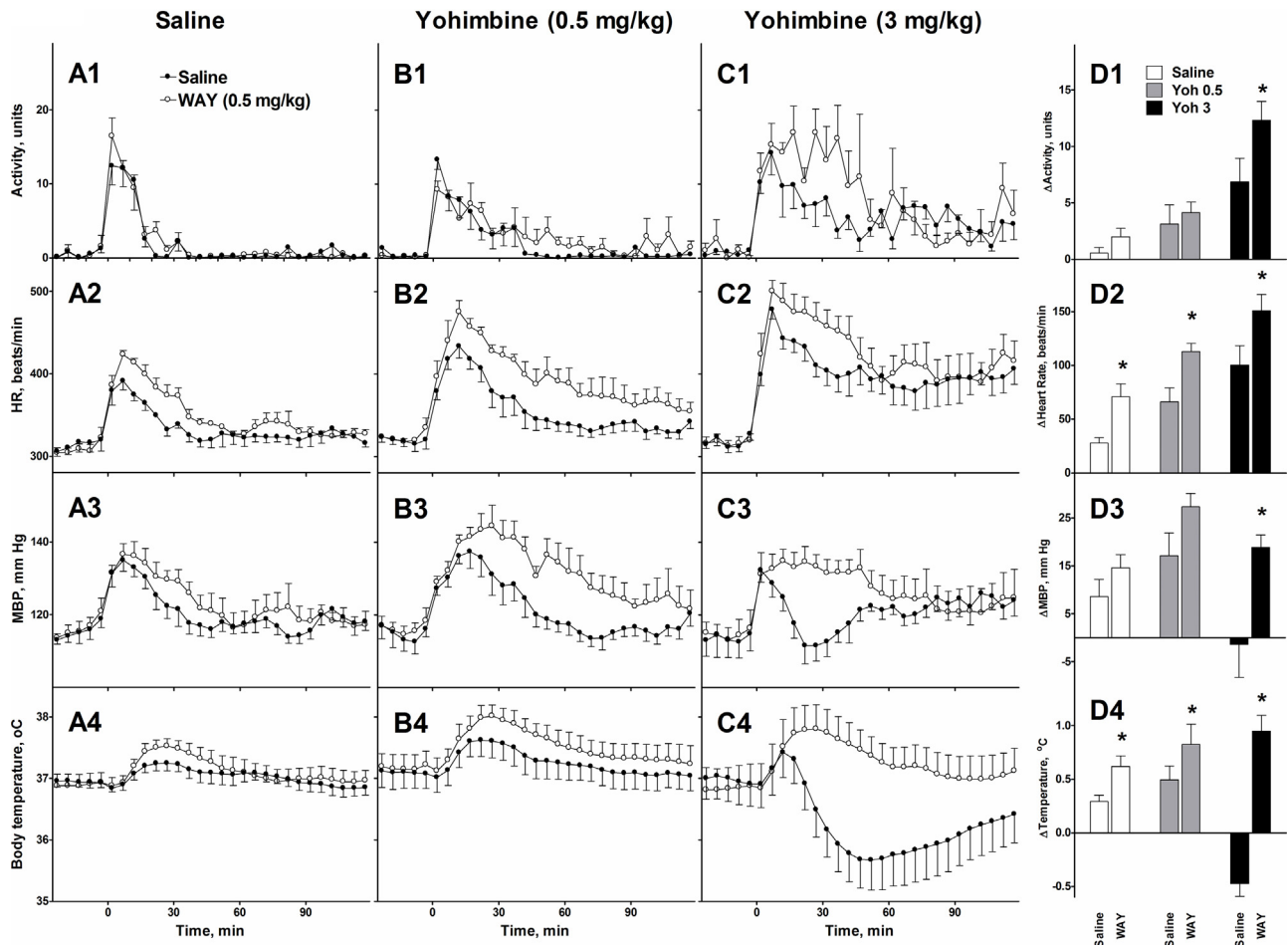


Fig. 2. Physiological responses to intraperitoneal injection of saline or WAY (0.5 mg/kg, i.p.) at time 0 followed by i.p. injection of saline or yohimbine (0.5 mg/kg or 3 mg/kg) at 5 min. The bar graphs (right column) show the averages of locomotor activity, cardiovascular parameters and core body temperature during the interval of 15–30 min after injection of yohimbine.

– Significant difference from saline pretreatment ($p < 0.05$).

$p < 0.001$; MBP $F(5,18) = 5.9$; $p = 0.002$; temperature $F(5,18) = 21.5$; $p < 0.001$). In low doses (0.5–2 mg/kg i.p.) yohimbine induced locomotor activation accompanied by tachycardia, mild hypertension, and a trend to a hyperthermia (Fig. 1). In higher doses (3 and 4.5 mg/kg) yohimbine reversed hypertension (Fig. 1C) and locomotor activation (Fig. 1A) but not tachycardia (Fig. 1B). The trend to increasing body temperature visible after 0.5 mg/kg was reversed by higher doses, and body temperature after 2 mg/kg was significantly lower than after 0.5 mg/kg, (Fig. 1D). In higher doses yohimbine induced dramatic hypothermia (Fig. 1D). Core body temperature fell to $34.7 \pm 0.6^\circ\text{C}$ after 3 mg/kg yohimbine and to $33.4 \pm 0.9^\circ\text{C}$ after 4.5 mg/kg yohimbine. The rates of decline in temperature between 5 and 30 min were similar after 3 mg/kg and 4.5 mg/kg yohimbine ($0.058 \pm 0.019^\circ\text{C}/\text{min}$ vs $0.058 \pm 0.004^\circ\text{C}/\text{min}$), but the nadir occurred later after the higher dose (59 ± 14 min vs 118 ± 19 min). Dose-response curve for yohimbine for temperature had a clear sigmoidal shape with EC_{50} equal 2.2 mg/kg (95% confidence interval 1.8–2.6 mg/kg).

Pretreatment with WAY (0.5 mg/kg, i.p.) had effects on all parameters (locomotion $F(1,12) = 10.9$; $p < 0.05$; HR $F(1,12) = 50.4$; $p < 0.001$; MBP $F(1,12) = 19.0$; $p < 0.001$; temperature $F(1,12) = 64.7$; $p < 0.001$) and effect on temperature was also dependent on the dose of yohimbine ($F(2,12) = 18.0$; $p < 0.001$). Administration of WAY did not affect the locomotor response to i.p. injection of saline or 0.5 mg/kg yohimbine, however it significantly increased locomotion after 3 mg/kg yohimbine (Fig. 2, A1–D1). Adminis-

tration of WAY moderately increased (i.e., by approximately 50 beats/min) tachycardia seen in response to saline and both 0.5 and 3.0 mg/kg yohimbine (Fig. 2, A2–D2). Unlike heart rate, the effect of WAY on blood pressure changes induced by yohimbine was dependent on the dose of yohimbine (Fig. 2, A3–D3). Increase of blood pressure induced by 0.5 mg/kg yohimbine was modified not significantly by WAY, but pretreatment with the antagonist of 5-HT_{1A} receptors clearly prevented a drop of blood pressure after 3 mg/kg yohimbine (Fig. 2, C3–D3). Finally, the 5-HT_{1A} antagonist slightly increased hyperthermic response to injections of saline or 0.5 mg/kg yohimbine (Fig. 2, A4–D4). However, administration of WAY completely abolished hypothermia evoked by 3 mg/kg yohimbine (Fig. 2, C4–D4).

4. Discussion

In many studies, in which yohimbine was used as a prototypical α_2 -adrenoblocker, doses of drug were significantly higher than required for specific effects of this compound on the α_2 -adrenoreceptor. Our data unequivocally demonstrated that high doses of yohimbine activate 5HT_{1A} receptors.

Administration of yohimbine at high doses results in significant hypothermia, similar to decrease of body temperature evoked by 8-hydroxy-2-(di-n-propylamino) tetralin (8-OH-DPAT), a prototypical 5-HT_{1A} agonist [17]. Yohimbine-induced hypothermia is prevented by WAY (our data and [32]) in a dose specific for

5-HT_{1A} blocking action [18]. Previously, we demonstrated that hypothermia evoked by systemic administration of the 8-OH-DPAT is mediated by activation of 5-HT_{1A} receptors in the ventromedial medulla [39]. Most likely, the effect of systemically-administered yohimbine on core body temperature is also mediated by the inhibition of ventromedial medulla through activation of 5-HT_{1A} receptors: the rate of cooling after high dose of yohimbine (0.06 ± 0.02 °C/min after 3 mg/kg yohimbine) is similar to one induced by inhibition of neuronal activity in ventromedial medulla [52].

What is the dose of yohimbine which can be clearly identified as evoking 5-HT_{1A} effect, using body temperature as an experimental end-point? Administration of 3 mg/kg results in clear hypothermia. Considering that the slope of temperature decline after 3 mg/kg is similar to the one after 4.5 mg/kg, the effect of yohimbine on thermoregulation is saturated at 3 mg/kg. The administration of 2 mg/kg results in significantly lower body temperature compared with lowest studied dose (0.5 mg/kg). In fact, if there is a progressive increase of hyperthermic action with increasing dose, then hypothermic effect of 1 mg/kg would simply be masked. Non-linear regression of dose-dependency of the hypothermic effects of yohimbine results in EC₅₀ estimate of 2.2 mg/kg. Therefore, we conclude that in doses above 1 mg/kg yohimbine has considerable 5-HT_{1A} agonistic activity when administered intraperitoneally. This estimation is supported by complete suppression of serotonergic neurons in the dorsal raphe nucleus by 0.5 mg/kg yohimbine intravenously, which could be reversed by WAY [32].

The data in the National Institute of Mental Health–Psychoactive Drug Screening Program (NIMH-PDSP) database reveal, that in rats yohimbine is approximately 80-fold more selective for alpha2-adrenergic receptors than to 5-HT_{1A} receptors. This selectivity corresponds to the ratio of doses needed to activate 5-HT_{1A} receptors to doses required to block alpha2-adrenoreceptors in conscious rats. Is it possible that WAY blocks hypothermic effects of yohimbine through receptors other than 5-HT_{1A}? WAY does not affect alpha1-adrenoreceptor-mediated responses in doses below 1 mg/kg [49], and the sensitivity of alpha2-adrenoreceptors to WAY is at least twice less than that of alpha1-adrenoreceptors (PDSP database). According to PDSP database the only other candidate receptor, to which both substances could bind with reasonable affinity, is the D₂ receptor. However, the reported threshold dose in which WAY blocks effects on 5-HT_{1A} is 6 µg/kg [35], and the ratio of K_i for WAY acting on two receptors exceeds 1000 (0.24 nM for 5-HT_{1A} vs 370 nM for D₂, PDSP), than the dose in this study (0.5 mg/kg) is not sufficient to block effects on D₂.

Presence of 5-HT_{1A}-mediated effects of yohimbine in doses higher than 1 mg/kg can be found using other experimental end-points. Yohimbine-induced locomotion after 0.5 mg/kg was not affected by WAY, while the effect of 3 mg/kg was augmented by WAY. Considering that the administration of 8-OH-DPAT, an agonist of 5-HT_{1A} receptors, increases locomotion by itself [12,14], but suppresses locomotion induced by other stimuli [8,20], this double action can explain the relatively small locomotor responses to yohimbine [42] and its ability to suppress locomotion in behavioral paradigms [9].

In low doses yohimbine increased inferior cardiac nerve discharge [30] and caused tachycardia [25,38]. However, in higher doses yohimbine inhibited nerve discharge, and this inhibition was reversed by spiperone [30], an agent with 5-HT_{1A}-antagonist properties [28]. Similarly, we found that high dose of yohimbine caused the reversal of hypertonic response, and this reversal was completely prevented by pretreatment with WAY. Yohimbine has also other effects typical for 5-HT_{1A} agonists, such as release of ACTH and corticosterone [36], which was observed in rats only in doses higher than 1 mg/kg [45].

There are accumulating data on the importance of 5-HT_{1A} agonistic properties of yohimbine in experimental studies. Yohimbine (2.5–7.5 mg/kg) disrupted prepulse inhibition [37] and produced antinociception [43] in rats via the action at 5-HT_{1A} receptors but not at alpha2-adrenoreceptors. Ability of alpha2-adrenoblockers to affect 5-HT_{1A} receptors is not unique for yohimbine: BRL-44408 recognizes 5-HT_{1A} receptors along with being alpha2-adrenoreceptor antagonist [31].

Understanding dose-dependence of effects of yohimbine mediated by different receptors has a potential to affect interpretation of various phenomena related to the use of this drug. Yohimbine is known to reinstate methamphetamine [44], cocaine [15,27], and alcohol seeking [26] behaviors, and it is assumed that these actions result from a blockade of alpha2-adrenoreceptors [4,26]. However, the doses of yohimbine in all these studies (1.25–5 mg/kg) appear to be sufficient to activate 5-HT_{1A} receptors, while a dose less than 1 mg/kg was not effective in reinstating food-seeking behavior [33]. Considering that yohimbine is quickly accumulated in the brain but is eliminated with $t_{1/2}$ of 7.7 h [23], the need to use high doses cannot be justified by pharmacokinetics. A potential role of 5-HT_{1A} receptors is supported by the ability of WAY to attenuate cocaine-induced reinstatement [6,41].

Similarly, an antagonism of general anesthesia in rats also requires 1–2 mg/kg of yohimbine [22], while 0.25 mg/kg of yohimbine did not reverse the anesthesia in cats [48]. We conclude that in the doses, in which it is used in many experimental paradigms, yohimbine is also a 5-HT_{1A} agonist.

Author's contributions

D.V.Z. designed the experiments; D.V.Z. and M.V.Z. conducted the experiments; D.V.Z. wrote the manuscript; D.V.Z., M.V.Z., J.A.D., and D.E.R. edited the manuscript.

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References

- [1] J.M. Arthur, S.J. Casañas, J.R. Raymond, Partial agonist properties of rauwolscine and yohimbine for the inhibition of adenylyl cyclase by recombinant human 5-HT_{1A} receptors, *Biochem. Pharmacol.* 45 (1993) 2337–2341.
- [2] M.L. Bertholomey, T.L. Verplaetse, C.L. Czachowski, Alterations in ethanol seeking and self-administration following yohimbine in selectively bred alcohol-preferring (P) and high alcohol drinking (HAD-2) rats, *Behav. Brain Res.* 238 (2013) 252–258.
- [3] S. Bhalla, V. Rapolaviciute, A. Gulati, Determination of $\alpha(2)$ -adrenoreceptor and imidazoline receptor involvement in augmentation of morphine and oxycodone analgesia by agmatine and BMS182874, *Eur. J. Pharmacol.* 651 (2011) 109–121.
- [4] J.M. Bossert, N.J. Marchant, D.J. Calu, Y. Shaham, The reinstatement model of drug relapse: recent neurobiological findings, emerging research topics, and translational research, *Psychopharmacology (Berl.)* 229 (2013) 453–476.
- [5] C.A. Branch, M.M. Knuepfer, Adrenergic mechanisms underlying cardiac and vascular responses to cocaine in conscious rats, *J. Pharmacol. Exp. Ther.* 263 (1992) 742–751.
- [6] J.J. Burmeister, E.M. Lungren, K.F. Kirschner, J.L. Neisewander, Differential roles of 5-HT receptor subtypes in cue and cocaine reinstatement of cocaine-seeking behavior in rats, *Neuropsychopharmacology* 29 (2004) 660–668.
- [7] J.J. Cai, D.A. Morgan, W.G. Haynes, J.B. Martins, H.C. Lee, Alpha 2-Adrenergic stimulation is protective against ischemia-reperfusion-induced ventricular arrhythmias in vivo, *Am. J. Physiol. Heart Circ. Physiol.* 283 (2002) H2606–H2611.

- [8] R.J. Carey, A. Shanahan, E.N. Damianopoulos, C.P. Müller, J.P. Huston, Behavior selectively elicited by novel stimuli: modulation by the 5-HT_{1A} agonist 8-OHDPAT and antagonist WAY-100635, *Behav. Pharmacol.* 19 (2008) 361–364.
- [9] P. Chopin, S. Pellow, S.E. File, The effects of yohimbine on exploratory and locomotor behaviour are attributable to its effects at noradrenaline and not at benzodiazepine receptors, *Neuropharmacology* 25 (1986) 53–57.
- [10] L. Cleary, C. Vandeputte, J.R. Docherty, Investigation of postjunctional alpha-1 and alpha-2-adrenoceptor subtypes in vas deferens from wild-type and alpha(2A/D)-adrenoceptor knockout mice, *Br. J. Pharmacol.* 138 (2003) 1069–1076.
- [11] L.A. Degernes, T.J. Kreeger, R. Mandsager, P.T. Redig, Ketamine-xylazine anesthesia in red-tailed hawks with antagonism by yohimbine, *J. Wildl. Dis.* 24 (1988) 322–326.
- [12] C.T. Dourish, P.H. Hutson, G. Curzon, Low doses of the putative serotonin agonist 8-hydroxy-2-(di-n-propylamino) tetralin (8-OH-DPAT) elicit feeding in the rat, *Psychopharmacology (Berl.)* 86 (1985) 197–204.
- [13] J.C. Doxey, A.C. Lane, A.G. Roach, N.K. Virdee, Comparison of the alpha-adrenoceptor antagonist profiles of idazoxan (RX 781,094), yohimbine, rauwolfscine and corynanthine, *Naunyn Schmiedeberg's Arch. Pharmacol.* 325 (1984) 136–144.
- [14] J.L. Evenden, K. Angeby-Möller, Effects of 8-hydroxy-2-(di-n-propylamino) tetralin (8-OH-DPAT) on locomotor activity and rearing of mice and rats, *Psychopharmacology (Berl.)* 102 (1990) 485–491.
- [15] M.W. Feltenstein, R.E. See, Potentiation of cue-induced reinstatement of cocaine-seeking in rats by the anxiogenic drug yohimbine, *Behav. Brain Res.* 174 (1) (2006) 1–8.
- [16] M.R. Goldberg, D. Robertson, Yohimbine: a pharmacological probe for study of the alpha 2-adrenoceptor, *Pharmacol. Rev.* 35 (1983) 143–180.
- [17] G.M. Goodwin, A.R. Green, A behavioural and biochemical study in mice and rats of putative selective agonists and antagonists for 5-HT₁ and 5-HT₂ receptors, *Br. J. Pharmacol.* 84 (1985) 743–753.
- [18] G. Griebel, R.J. Rodgers, G. Perrault, D.J. Sanger, The effects of compounds varying in selectivity as 5-HT(1A) receptor antagonists in three rat models of anxiety, *Neuropharmacology* 39 (2000) 1848–1857.
- [19] Y. Hikasa, K. Masuda, Y. Asakura, Y. Yamashita, C. Sato, M. Kamio, A. Miura, T. Taniguchi, N. Minamizuru, Identification and characterization of platelet α 2-adrenoceptors and imidazoline receptors in rats, rabbits, cats, dogs, cattle, and horses, *Eur. J. Pharmacol.* 720 (2013) 363–375.
- [20] V. Hillegaart, M.L. Wadenberg, S. Ahlenius, Effects of 8-OH-DPAT on motor activity in the rat, *Pharmacol. Biochem. Behav.* 32 (1989) 797–800.
- [21] L.C. Houtepen, D.P. Peterse, K.G. Westphal, B. Olivier, C.H. Vinkers, The autonomic stress-induced hyperthermia response is not enhanced by several anxiogenic drugs, *Physiol. Behav.* 102 (2011) 105–109.
- [22] W.H. Hsu, S.I. Bellin, H.D. Dellmann, C.E. Hanson, Xylazine-ketamine-induced anesthesia in rats and its antagonism by yohimbine, *J. Am. Vet. Med. Assoc.* 189 (1986) 1040–1043.
- [23] J.W. Hubbard, S.L. Pfister, A.M. Biediger, T.C. Herzig, T.K. Keeton, The pharmacokinetic properties of yohimbine in the conscious rat, *Naunyn Schmiedeberg's Arch. Pharmacol.* 337 (1988) 583–587.
- [24] T.J. Kreeger, U.S. Seal, Immobilization of coyotes with xylazine hydrochloride-ketamine hydrochloride and antagonism by yohimbine hydrochloride, *J. Wildl. Dis.* 22 (1986) 604–606.
- [25] W.J. Lang, G.A. Lambert, M.L. Rush, The role of the central nervous system in the cardiovascular responses to yohimbine, *Arch. Int. Pharmacodyn. Ther.* 217 (1975) 57–67.
- [26] A.D. Lê, S. Harding, W. Juzysch, D. Funk, Y. Shaham, Role of alpha-2 adrenoceptors in stress-induced reinstatement of alcohol seeking and alcohol self-administration in rats, *Psychopharmacology (Berl.)* 179 (2005) 366–373.
- [27] B. Lee, S. Tiefenbacher, D.M. Platt, R.D. Spearman, Pharmacological blockade of alpha-2-adrenoceptors induces reinstatement of cocaine-seeking behavior in squirrel monkeys, *Neuropsychopharmacology* 29 (2004) 686–693.
- [28] J.T. Lum, M.F. Piercey, Electrophysiological evidence that spiperone is an antagonist of 5-HT_{1A} receptors in the dorsal raphe nucleus, *Eur. J. Pharmacol.* 149 (1988) 9–15.
- [29] T. Matsunaga, H. Tsukada, S. Nishiyama, Y. Sekine, T. Kakiuchi, M. Iyo, N. Mori, Yohimbine increases the binding potential for [¹¹C]flumazenil in the monkey brain, *J. Neural. Transm.* 108 (2001) 1375–1382.
- [30] R.B. McCall, L.T. Harris, K.A. King, Sympatholytic action of yohimbine mediated by 5-HT_{1A} receptors, *Eur. J. Pharmacol.* 199 (1991) 263–265.
- [31] J.J. Meana, L.F. Callado, A. Pazos, B. Grijalba, J.A. García-Sevilla, The subtype-selective alpha 2-adrenoceptor antagonists BRL 44,408 and ARC 239 also recognize 5-HT_{1A} receptors in the rat brain, *Eur. J. Pharmacol.* 312 (1996) 385–388.
- [32] M.J. Millan, A. Newman-Tancredi, V. Audinot, D. Cussac, F. Lejeune, J.P. Nicolas, F. Cogé, J.P. Galizzi, J.A. Boutin, J.M. Rivet, A. Dekeyne, A. Gobert, Agonist and antagonist actions of yohimbine as compared to fluparoxan at alpha(2)-adrenergic receptors (AR) s serotonin (5-HT)(1A), 5-HT(1B), 5-HT(1D) and dopamine D(2) and D(3) receptors. Significance for the modulation of frontocortical monoaminergic transmission and depressive states, *Synapse* 35 (2000) 79–95.
- [33] S.G. Nair, S.M. Gray, U.E. Ghitz, Role of food type in yohimbine- and pellet-priming-induced reinstatement of food seeking, *Physiol. Behav.* 88 (2006) 559–566.
- [34] E.P. Nord, M.J. Howard, A. Hafezi, P. Moradeshagi, S. Vaystub, P.A. Insel, Alpha 2 adrenergic agonists stimulate Na⁺-H⁺ antiport activity in the rabbit renal proximal tubule, *J. Clin. Invest.* 80 (1987) 1755–1762.
- [35] M.F. O'Neill, G.J. Sanger, GR46611 potentiates 5-HT_{1A} receptor-mediated locomotor activity in the guinea pig, *Eur. J. Pharmacol.* 370 (1999) 85–92.
- [36] P. Osei-Owusu, A. James, J. Crane, K.E. Scrogin, 5-Hydroxytryptamine 1A receptors in the paraventricular nucleus of the hypothalamus mediate oxytocin and adrenocorticotropin hormone release and some behavioral components of the serotonin syndrome, *J. Pharmacol. Exp. Ther.* 313 (2005) 1324–1330.
- [37] S.B. Powell, J. Palomo, B.S. Carasso, V.P. Bakshi, M.A. Geyer, Yohimbine disrupts prepulse inhibition in rats via action at 5-HT_{1A} receptors, not alpha-2-adrenoceptors, *Psychopharmacology (Berl.)* 180 (2005) 491–500.
- [38] A.R. Rahman, F.M. Sharabi, Presynaptic alpha receptors in relation to the cardiovascular effect of yohimbine in the anesthetized cat, *Arch. Int. Pharmacodyn. Ther.* 252 (1981) 229–240.
- [39] D.E. Rusyniak, M.V. Zaretskaia, D.V. Zaretsky, J.A. DiMicco, 3,4-Methylenedioxymethamphetamine- and 8-hydroxy-2-di-n-propylamino-tetralin-induced hypothermia: role and location of 5-hydroxytryptamine 1A receptors, *J. Pharmacol. Exp. Ther.* 323 (2007) 477–487.
- [40] B. Scatton, B. Zivkovic, J. Dedek, Antidopaminergic properties of yohimbine, *J. Pharmacol. Exp. Ther.* 215 (1980) 494–499.
- [41] S. Schenk, Effects of the serotonin 5-HT(2) antagonist, ritanserin, and the serotonin 5-HT(1A) antagonist, WAY 100,635, on cocaine-seeking in rats, *Pharmacol. Biochem. Behav.* 67 (2000) 363–369.
- [42] B.E. Schroeder, C.A. Schiltz, A.E. Kelley, Neural activation profile elicited by cues associated with the anxiogenic drug yohimbine differs from that observed for reward-paired cues, *Neuropsychopharmacology* 28 (2003) 14–21.
- [43] H.E. Shannon, E.A. Lutz, Yohimbine produces antinociception in the formalin test in rats: involvement of serotonin(1A) receptors, *Psychopharmacology (Berl.)* 149 (2000) 93–97.
- [44] J.D. Shepard, J.M. Bossert, S.Y. Liu, Y. Shaham, The anxiogenic drug yohimbine reinstates methamphetamine seeking in a rat model of drug relapse, *Biol. Psychiatry* 55 (2004) 1082–1089.
- [45] K. Shimizu, Effect of alpha 1- and alpha 2-adrenoceptor agonists and antagonists on ACTH secretion in intact and in hypothalamic deafferented rats, *Jpn. J. Pharmacol.* 36 (1984) 23–33.
- [46] R.J. Smith, G. Aston-Jones, Alpha(2) Adrenergic and imidazoline receptor agonists prevent cue-induced cocaine seeking, *Biol. Psychiatry* 70 (2011) 712–719.
- [47] E.H. Stotz-Potter, S.M. Morin, J.A. DiMicco, Effect of microinjection of muscimol into the dorsomedial or paraventricular hypothalamic nucleus on air stress-induced neuroendocrine and cardiovascular changes in rats, *Brain Res.* 742 (1996) 219–224.
- [48] J. Versteegen, X. Fargetton, S. Zanker, I. Donnay, F. Ectors, Antagonistic activities of atipamezole, 4-aminopyridine and yohimbine against medetomidine/ketamine-induced anaesthesia in cats, *Vet. Rec.* 128 (1991) 57–60.
- [49] R. Villalobos-Molina, J.J. Lopez-Guerrero, I.A. Gallardo-Ortiz, M. Ibarra, Evidence that the hypotensive effect of WAY 100,635, a 5-HT_{1A} receptor antagonist, is related to vascular alpha 1-adrenoceptor blockade in the adult rat, *Auton. Autacoid Pharmacol.* 22 (2002) 171–176.
- [50] J.C. Winter, R.A. Rabin, Antagonism of the stimulus effects of yohimbine and 8-hydroxydipropylaminotetralin, *Pharmacol. Biochem. Behav.* 44 (1993) 851–855.
- [51] J.C. Winter, R.A. Rabin, Yohimbine as a serotonergic agent: evidence from receptor binding and drug discrimination, *J. Pharmacol. Exp. Ther.* 263 (1992) 682–689.
- [52] D.V. Zaretsky, M.V. Zaretskaia, J.A. DiMicco, Stimulation and blockade of GABA(A) receptors in the raphe pallidus: effects on body temperature, heart rate, and blood pressure in conscious rats, *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 285 (2003) R110–R116.