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Cardiovascular stimulant actions of bupropion in comparison to cocaine in the rat

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Bupropion
(PubChem CID: 444)
Cocaine
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Desipramine
(PubChem CID: 65327)
Methoxamine
(PubChem CID: 6081)
Modafinil
(PubChem CID: 4236)
Nifedipine
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Noradrenaline
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ABSTRACT

Stimulants are banned in competition by the World Anti-Doping Agency, except for a small number of therapeutic agents subject to monitoring, including bupropion. We have examined the potency of bupropion in comparison with two agents banned in competition, adrafinil and modafinil, and with cocaine and desipramine as blockers of the noradrenaline re-uptake transporter in peripheral tissues of the rat. For studies in vivo, the pressor response to noradrenaline in the anaesthetized rat was studied. Cocaine, desipramine and bupropion at doses of 0.1, 0.3 and 1 mg/kg, respectively, significantly increased the pressor response to noradrenaline. Overall, cocaine and desipramine were approximately 2–5 times more potent than bupropion in vivo in the rat. Adrafinil and modafinil (both 3 mg/kg) did not significantly affect the pressor response. Bupropion was chosen for further study. In 1 Hz paced rat right ventricular strips, bupropion (30 μM) significantly increased the potency of noradrenaline at increasing the force of contraction. In rat vas deferens, bupropion and cocaine produced concentration-dependent increases in the contractile response to nerve stimulation, and cocaine was 11 times more potent than bupropion. Since bupropion is used clinically in doses of up to 300 mg, it is likely that bupropion has actions at the noradrenaline transporter, and thus cardiovascular stimulant actions, in clinical doses. This may explain findings of increased exercise performance with bupropion.

1. Introduction

Stimulants are banned in competition by the World Anti-Doping Agency, and a large number of compounds have been placed on the Prohibited List (WADA, 2011). However, a number of agents available as over the counter medicines for therapeutic uses, although subject to monitoring, may enhance performance (see Docherty, 2008). Among these is bupropion. Bupropion is reported to be a weak antidepressant, acting as a weak blocker of the monoamine re-uptake transporters, is widely available for the treatment of tobacco dependence (Hays and Ebbert, 2003) and has been shown to improve attention (Acheson and de Wit, 2008). Bupropion as a dopamine/noradrenaline re-uptake inhibitor has been reported to enhance exercise performance in the heat (Watson et al., 2005; Roelands et al., 2009) but only at the maximum therapeutic dose of 300 mg (Roelands et al., 2012), although the mechanism of action has not been fully established. These findings may be relevant to competition, as it has been reported that the number of antidepressant findings, among these bupropion, in elite sport approximately doubled in Germany from 2005 to 2008 (Machnik et al., 2009).

Bupropion has been studied particularly for its central and dopaminergic actions, but it is also of interest in sports physiology in terms of its peripheral cardiovascular actions. Relatively little is know of its potency as a peripheral stimulant. Bupropion (150 mg) has been reported to raise blood pressure in man (Martins et al.,

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2009; Thase et al., 2008), and bupropion produces tachycardia and hypertension in overdose (Balit et al., 2003).

We have compared the actions of bupropion, with those of two CNS stimulant agents banned in competition, adrafinil and modafinil (see Docherty, 2008), in the anaesthetized rat preparation, and have examined in detail the potency of bupropion, in comparison with cocaine as blockers of the noradrenaline reuptake transporter in peripheral tissues of the rat, employing right ventricle and vas deferens.

Some of these results have been published in abstract form (Killian and Docherty, 2013).

2. Materials and methods

2.1. General

Male Wistar rats (250–300 g) were obtained from Trinity College Dublin. All studies have been approved by the Department of Health (Ireland) and by the RCSI Research Ethics Committee. Animals were killed by anaesthesia with pentobarbitone (50 mg/kg, i.p.) and cervical dislocation for in vitro studies, or anaesthetized with pentobarbitone for blood pressure recording.

2.2. Cardiovascular studies under anaesthesia.

Rats were anaesthetised with pentobarbitone (50 mg/kg, i.p., and maintenance doses, as required, i.v.). The carotid artery and jugular vein were cannulated for recording of blood pressure, and for the injection of drugs. At the end of the experiment, animal was killed by overdose of anaesthetic. Noradrenaline (1 μg/kg) or methoxamine (30 μg/kg) was given i.v. at 5 min intervals until consistent pressor responses (diastolic blood pressure) had been obtained. In a small number of experiments, control pressor responses of less than 20 mmHg were obtained and the dose of agonist was increased 3 fold to obtain a larger control pressor response in the range 20–60 mmHg. Vehicle, bupropion, adrafinil, modafinil, desipramine or cocaine were then administered in 2 or usually 3 doses at 5 min intervals and the noradrenaline or methoxamine pressor response was repeated 5 min after each dose. For adrafinil and modafinil, low potency and limited solubility meant that a single dose of 3 mg/kg was studied, and the drug was injected slowly over 1 min. In some bupropion experiments, following 3 doses of bupropion up to 10 mg/kg, cocaine (1 mg/kg) was added and the noradrenaline response repeated.

2.3. Rat vas deferens

Epididymal portions of rat vas deferens were obtained. Tissues were placed between platinum electrodes and attached to myograph transducers under 1 g tension in organ baths at 37 °C in Krebs–Henseleit solution of the following composition (mM): NaCl, 119; $NaHCO_3$, 25; D-glucose, 11.1; KCl, 4.7; $CaCl_2$, 2.5; KH_2PO_4, 1.2; $MgSO_4$, 1.0. The solution was bubbled with 5% CO_2/95% O_2.

Epididymal portions were stimulated every 5 min with a single stimulus (0.5 ms pulses, supramaximal voltage) using a Grass S88 stimulator to produce isometric contractions. Nifedipine (10 μM) was present in the Krebs–Henseleit solution to block the non-adrenergic component of the twitch (see Blakeley et al., 1981), and test agents (bupropion, cocaine or vehicle) were added in cumulative concentrations at 5 min intervals in the concentration range 0.1–10 μM (cocaine) or 1–100 μM (bupropion). An isometric twitch was obtained following 5 min exposure to each test agent concentration, or vehicle.

In addition to measuring the height of contractions to nerve stimulation, the duration of contraction was measured as follows. For the highest concentration of cocaine (10 μM) and bupropion (100 μM) and the equivalent vehicle, the duration of contraction was measured as the time from peak contraction to 10% of peak contraction.

2.4. Rat right ventricle

Rat heart was removed and strips of right ventricle (one or sometimes two strips per heart) were placed in Krebs–Henseleit solution of the following composition (in mM): NaCl, 119; $NaHCO_3$, 25; D-glucose, 11.1; KCl, 4.7; $CaCl_2$, 2.5; KH_2PO_4, 1.2; $MgSO_4$, 1.0. Strips of right ventricle were set up between platinum electrodes and isometric force transducers under 2 g tension and paced at a frequency of 1 Hz (supramaximal voltage, 0.5 ms pulses) using a Grass S88 stimulator. Vessels were allowed to equilibrate at 37 °C and were gassed with 5% CO_2 in O_2.

Following 30-min equilibration, responsiveness was tested with noradrenaline (10 μM). One hour later, a preliminary concentration–response curve to noradrenaline was carried out in 0.5 log unit increments beginning with 1 nM, until a maximum response was reached. This preliminary concentration–response curve served to sensitise the tissues, and was not used in calculations. Bathing fluid was then changed every 15 min for the next hour. Tissues were then exposed to test agent or vehicle for 30 min, and the concentration–response curve to noradrenaline was then repeated in the continuing presence of test agent or vehicle.

2.5. Statistics

Values are expressed as mean and standard error of mean (SEM), with n, the number of animals. Effects of test drugs against agonist or nerve-evoked responses were compared with those of vehicle. pEC_{50} values (in vitro) or log ED_{50} values (in vivo) for noradrenaline or test agents were calculated by non-linear regression analysis using the GraphPad Prism programme for MacIntosh. Maximum contractions were measured in grams weight, and blood pressure in mmHg. Maximum contractions or pressor responses in the presence of test agents or vehicle were expressed as a percentage of initial response. Differences between groups and vehicle were compared using the Instat programme for Macintosh by analysis of variance, with Dunnett test for comparison of effects of vehicle with test drug. Means were considered significantly different when P values were <0.05.

2.6. Drugs

Adrafinil (Sigma, Ireland); Bupropion (Sigma); cocaine hydrochloride (Sigma); desipramine hydrochloride (Sigma); methoxamine hydrochloride (Sigma); modafinil (Tocris Bioscience, Bristol, UK); (±)-noradrenaline hydrochloride (Sigma), nifedipine (Sigma).

All drugs were dissolved in distilled water, except for adrafinil and modafinil, which were dissolved in ethanol (100%) and diluted in distilled water. Maximum injected dose of ethanol intravenously was of 30%, injection slowly over 1 min in a volume of 1 ml/kg. Higher doses affected responses to noradrenaline.

3. Results

3.1. Anaesthetized rat

In anaesthetized rats, resting diastolic blood pressure (DBP) was 93.4 ± 2.6 mmHg ($n=29$). Noradrenaline (1 μg/kg) produced a pressor (DBP) response of 29.8 ± 2.3 mmHg ($n=29$). Baseline DBP