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Nicotine's central cardiovascular actions: Receptor subtypes involved and their possible physiological role in anaesthetized rats

Christopher Moore [a,*], Yun Wang [b], Andrew G. Ramage [a,*]

[a] UCL Research Department of Neuroscience, Physiology and Pharmacology, Division of Biosciences, University College London, Gower St., London WC1E 6BT UK
[b] Institutes of Brain Science and State Key Laboratory of Medical Neurobiology, Fudan University, Shanghai 200032, China

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ABSTRACT

Centrally applied nicotine causes changes in blood pressure and vasopressin release. The involvement of different neuronal nicotinic receptor subtypes in these actions was investigated in anaesthetized rats. Nicotine administered i.c.v. caused a dose-related increase in blood pressure and renal sympathoinhibition, while i.c. administration also caused a bradycardia. In the presence of the V_{1A} receptor antagonist (i.v.), nicotine (i.c) now caused a depressor response along with sympathoinhibition and bradycardia. Nicotine (0.3 μmol/kg; i.c.v.) in the presence of the α4β2 receptor antagonist, dihydro-β-erythroidine, (i.c.v.) evoked renal sympathoexcitation, while the α7 receptor antagonist, methyllycaconitine, delayed the expected sympathoinhibition. Both receptor antagonists blocked the pressor response. Dihydro-β-erythroidine (i.c., 10 μmol/kg) alone caused a transient pressor response and increased renal nerve activity. Methyllycaconitine (i.c., 0.1 μmol/kg) alone caused a slow fall in blood pressure and renal nerve activity, while the higher doses caused a pressor response and increased renal nerve activity. It was concluded that for nicotine to release vasopressin, activation of both α4β2 and α7 receptors is required. The ability of nicotine to cause sympathoinhibition is mediated by β4*-containing receptors, possibly α3β4 receptors, and that activation of these receptors can override the sympathoexcitatory action of α4β2 and α7 receptors. The ability of dihydro-β-erythroidine and high doses of methyllycaconitine i.c. to cause sympathoexcitation and a pressor response is due to receptor antagonists blocking these sympathoinhibitory β4*-containing receptors, which receive a tonic cholinergic input. As the low dose of methyllycaconitine caused sympathoinhibition, this indicates that sympathoexcitatory α7 receptors also receive a tonic input.

1. Introduction

Nicotine has long been known to have cardiovascular actions (Bisset et al., 1975) either directly or via the release of vasopressin into the circulation (Burn et al., 1945). It has previously been shown that of the possible 5 neuronal nicotinic acetylcholine receptor subunits, α2*, α3*, α4*, α6* and α7 (see Albuquerque et al., 2009; Colquhoun et al., 2003), the α4β2 and α7 neuronal nicotinic acetylcholine receptors, at the level of the medulla, cause a pressor response and renal sympathoexcitation (Moore et al., 2008). The present experiments were carried out to determine whether the central cardiovascular actions of nicotine can be explained by just the activation of α4β2 and α7 neuronal nicotinic acetylcholine receptors or whether additional neuronal nicotinic acetylcholine receptors were involved. This was carried out by examining the effects of nicotine, given centrally by intracerebroventricular (i.c.v.) injection in the presence of dihydro-β-erythroidine (DhβE; α4β2 receptor antagonist) and methyllycaconitine (MLA; α7 receptor antagonist) at concentrations previously shown to block their respective receptors (Moore et al., 2008). In addition, the effects of vasopressin release on nicotine's central cardiovascular actions were investigated by studying the affect of nicotine given by intracisternal (i.c.) injection in the presence and absence of the selective vasopressin V_{1A} receptor antagonist ([β-Mercapto-β,β-Cyclopentamethylenepropionyl1,O-Me-Tyr2, Arg8]-Vasopressin, (d(CH2)5Tyr(Me)AVP)) given i.v. Finally, experiments were carried out to determine whether α4β2 and α7 neuronal nicotinic acetylcholine receptors at the level of the medulla are involved in the maintenance of resting blood pressure and sympathetic nerve activity by giving DhβE and MLA alone i.c. Preliminary communication of some of these data has been previously given (Moore et al., 2006).

2. Materials and methods

2.1. Animal preparation, central cannulation and recording of renal sympathetic nerve activity

Experiments were performed on 85 male Sprague–Dawley rats (250–350 g). Anaesthesia was induced by isoflurane (2.5% in oxygen)

* Corresponding author at: UCL Research Department of Neuroscience, Physiology and Pharmacology, (P O. Box 34) Gower St., London WC1E 6BT, UK.
E-mail address: chris.moore@ucl.ac.uk (C. Moore).

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and maintained with α-chloralose (100 mg/kg, i.v.). Supplementary doses of α-chloralose (10–20 mg/kg, i.v.) were given as required. Depth of anaesthesia was assessed by the stability of cardiovascular and respiratory variables being recorded. The right carotid artery was cannulated by the measurement of blood pressure and for sampling arterial blood for analysis of pH and blood gases. Blood pressure was measured using a pressure transducer (Gould Statham P23XL) and the heart rate was derived electronically from the blood pressure signal (Gould Biotach Amplifier). The left jugular vein was cannulated for drug administration and a tracheal cannula was implanted. Body temperature was monitored by a rectal probe and maintained at 36–38 °C with a homoeothermic blanket system (Harvard). The animals were artificially ventilated (rate 50 strokes/min, stroke volume 8 ml/kg) with oxygen enriched room air by the use of a positive pressure pump (Harvard Rodent Ventilator 683) and neuromuscular blockage was produced with decamethonium (3 mg/kg, i.v.). Blood samples were taken from a T-piece on the carotid arterial cannula and blood gases and pH were monitored with a Corning pH/blood gas analyzer. Blood gases were maintained between 90–130 mm Hg PO_2, 40–50 mm Hg $PC0_2$ and pH 7.3–7.4. Adjustments of the respiratory pump volume were made as necessary to maintain blood gas and pH balance. Once ventilated, the animals were infused (6 ml/kg/h) into the jugular vein with a solution comprising of 10 ml plasma substitute (gelofusine), 10 ml distilled water, 40 mg glucose, 168 mg sodium bicarbonate and 10 mg decamethonium. This is to prevent the development of non-respiratory acidosis and to maintain blood volume and neuromuscular blockade. During neuromuscular blockade, the depth of anaesthesia was assessed by monitoring the stability of the arterial blood pressure and heart rate and the cardiovascular responses to pinching the paws.

2.2. Cannulation of the lateral cerebral ventricle and cisterna magna

The rats were placed in a stereotaxic head holder and a stainless steel guide cannula (22 gauge) was implanted into the right lateral cerebral ventricle. The coordinates used from the bregma were 4 mm ventral, 1.5 mm lateral and 1 mm posterior. For the cisterna magna the atlanto-occipital membrane was exposed. A stainless steel guide cannula (23 gauge) was inserted perpendicularly into the membrane to the depth of its bevel. Drugs and vehicle solution were administered through an i.c. injection cannula (28 gauge) attached by a length of polythene tubing to a 25 μl syringe (Hamilton). Successful cannulation was verified by the filling of the stainless steel guide cannula with cerebrospinal fluid and at the end of the experiment, by the administration of 5 μl of 2% pontamine sky blue dye.

2.3. Recording of renal nerve activity

The left kidney was exposed by a retroperitoneal approach and was deflected laterally to reveal the renal artery and nerve. The nerve was cleared of connective tissue and positioned on a bipolar silver hook electrode. The renal nerve activity was amplified (Digitimer NL104), filtered (Digitimer NL125, frequency band 100–500 Hz) and quantified by integrating the signal above the background noise over 5 s with a solid state integrator (Medical Electronics workshop, Royal Free Hospital School of Medicine). At the end of the experiment 20 mg of sodium pentobarbital (per animal) was used to reduce nerve activity to zero to validate the integrator threshold. At the beginning of each experiment the baroreceptor reflex response was tested by observing whether renal nerve activity and heart rate were reduced by a rise in blood pressure caused by noradrenaline (25 ng per animal, i.v.) and were raised by a reduction in blood pressure caused by sodium nitroprusside (0.6 μg per animal, i.v.) only preparations with an intact baroreceptor reflex was used.

2.4. Experimental protocol

The preparation was allowed to stabilise for 20 min before the administration of 5 μl saline (i.c.v. and i.c.) was given (saline flush). Ten minutes after the initial saline flush a single dose of drug or saline control was given i.c.v. for i.c. and the response was followed for 20 min. In receptor antagonist i.c.v. studies, receptor antagonists or saline was administered i.c.v. 3 min before injection of test drug or saline (i.c.v.) and the response followed for 20 min. For the V_{1A} receptor $d(CH_2)_5$Tyr(Me)AVP antagonist i.v. experiments this receptor antagonist was given 5 min after the initial flush and 10 min later nicotine or saline i.c. and the response was followed for 20 min. In the receptor antagonists i.c. alone studies, ten minutes after the initial saline flush a single dose of drug or saline control was given i.c. and the response was followed for 20 min. In each rat the cardiovascular response of a single dose of the nicotine or saline was recorded.

2.5. Statistical analysis

Baseline values were taken 1 min before the addition of the drug or vehicle. Drug administration starts at time zero and ends 15 s later. All results are expressed as changes from baseline values. Nerve activity was measured as the average of the integrated values over 10 s for the first 5 min and then as an average of the integrated values over 1 min in arbitrary units and was expressed as the percentage change from baseline. Changes in mean blood pressure, heart rate and renal nerve activity caused by the test drug were compared with time-matched vehicle controls using two-way analysis of variance and were subsequently analysed using the least significant difference test. All values are expressed as the mean ± S.E.M; differences in the mean were taken as significant when $P<0.05$.

2.6. Drugs and solutions

Drugs were obtained from the following sources: α-chloralose, sodium nitroprusside; decamethonium bromide, nicotine hydrogen tartrate, methyllycaconitine citrate, [β-Mercapto-β,β-Cyclopentamethylenepropionyl^1,O-Me-Tyr^2,Arg^8]-Vasopressin, ($d(CH_2)_5$Tyr(Me)AVP); from Sigma Chemical Co., Poole, Dorset, U.K.; dihydro-β-erythroidine from Tocris Cookson Ltd, Avonmouth, Bristol, UK.; noradrenaline acid tartrate from Winthrop, Guildford, Surrey, U.K.; isoflurane from Abbott Labs Ltd, Queenborough, Kent, U.K.; Gelofusine from Braun Medical Ltd, Aylesbury, Bucks, U.K. Drugs given i.c.v. and i.c. were dissolved in 0.9% w/v saline. Solutions were administered in dose volume of 5 μl over a 15 s period. All drugs given i.v., i.c.v. and i.c. were dissolved in saline.

Drug/molecular target nomenclature conforms to IUPHAR LGIC nomenclature (Collingridge et al. (2009). A nomenclature for ligand-gated ion channels. Neuropharmacology. 56 (1): 2–5 and Neubig et al. (2003) International Union of Pharmacology Committee on Receptor Nomenclature and Drug Classification. XXXVIII. Update on terms and symbols in quantitative pharmacology. Pharmacol Rev. 55 (4): 597–606).

3. Results

3.1. Saline controls

In saline (i.c.v. and i.c.) pre-treated animals, saline injected i.c.v. and i.c. (5 μl, n = 5, saline control) had no effect on mean arterial blood pressure, heart rate or renal nerve activity and these variables remained stable for the duration of the experiment (see Fig. 3). The baseline values for mean arterial blood pressure and heart rate before saline i.c.v. and i.c pre-treatment were 137 ± 14 mm Hg and 380 ± 27 beats/min and 112 ± 13 mm Hg and 385 ± 32 beats/min respectively. In saline controls (i.c.v., n = 5) for receptor antagonist studies,