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RESEARCH PAPER

The role of dopamine D_2, but not D_3 or D_4, receptor subtypes, in quinpirole-induced inhibition of the cardioaccelerator sympathetic outflow in pithed rats

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BACKGROUND AND PURPOSE

Quinpirole (a dopamine D_2-like receptor agonist) inhibits the cardioaccelerator sympathetic outflow in pithed rats by sympathoinhibitory D_2-like receptors. The present study was designed to identify pharmacologically the specific D_2-like receptor subtypes (i.e. D_2, D_3 and D_4) involved in this sympathoinhibition by quinpirole.

EXPERIMENTAL APPROACH

One hundred fourteen male Wistar rats were pithed, artificially ventilated with room air and prepared for either preganglionic spinal (C_7-T_1) stimulation of the cardioaccelerator sympathetic outflow (n = 102) or i.v. bolus injections of exogenous noradrenaline (n = 12). This approach resulted in frequency-dependent and dose-dependent tachycardic responses, respectively, as previously reported by our group.

KEY RESULTS

I.v. continuous infusions of quinpirole (0.1–10 μg kg^{-1} min^{-1}), but not of saline (0.02 mL min^{-1}), dose-dependently inhibited the sympathetically induced tachycardic responses. Moreover, the cardiac sympathoinhibition induced by 3 μg kg^{-1} min^{-1} quinpirole (which failed to affect the tachycardic responses to i.v. noradrenaline) was: (i) unchanged after i.v. injections of the antagonists SB-277011-A (D_3; 100–300 μg kg^{-1}) or L-745,870 (D_4; 30–100 μg kg^{-1}); and (ii) markedly blocked and abolished by, respectively, 100 and 300 μg kg^{-1} of the D_2 preferring receptor subtype antagonist L-741,626. These doses of antagonists, which did not affect *per se* the sympathetically induced tachycardic responses, were high enough to completely block their respective receptors.

CONCLUSIONS AND IMPLICATIONS

The cardiac sympathoinhibition induced by 3 μg kg^{-1} min^{-1} quinpirole involves the dopamine D_2 receptor subtype, with no evidence for the involvement of the D_3 or D_4 subtypes. This provides new evidence for understanding the modulation of the cardioaccelerator sympathetic outflow.

Abbreviations

DMSO, dimethyl sulphoxide; D-R curves, dose-response curves; L-741,626, (±)-3-[4-(4-Chlorophenyl)-4-hydroxypiperidinyl]methylindole; L-745,870, 3-[[4-(4-Chlorophenyl)piperazin-1-yl]methyl]-1H-pyrrolo[2,3-b]pyridine hydrochloride; SB-277011-A, {trans-N-[4-[2-(6-cyano-1,2,3,4-tetrahydroisoquinolin-2-yl)ethyl]cyclohexyl]-4-quinolininecarboxamide}; S-R curves, stimulus-response curves

Introduction

Dopamine, the immediate metabolic precursor of noradrenaline and adrenaline, modulates multiple physiological functions by interacting with receptors located centrally and peripherally (Willems *et al.*, 1985; Missale *et al.*, 1998; Jose *et al.*, 2003). With the conjunction of structural, transductional and operational (pharmacological) criteria, dopamine receptors can be classified into D_1-like (which includes the D_1 and D_5 subtypes and activates G_s proteins) and D_2-like (which includes the D_2, D_3 and D_4 subtypes and activates $G_{i/o}$ proteins) types (see Missale *et al.*, 1998; Neve *et al.*, 2004; receptor nomenclature follows Alexander *et al.*, 2011).

Dopamine produces complex responses in the cardiovascular system by its capability to interact with α- and β-adrenoceptors as well as dopamine receptors (Goldberg, 1972; Missale *et al.*, 1998; Beaulieu and Gainetdinov, 2011). D_1-like receptors are mainly located on blood vessels, the heart and the kidneys inducing direct vasodilatation, cardiac stimulation and natriuresis on the proximal tubules, respectively; whereas D_2-like receptors are mostly found prejunctionally on perivascular and cardiac sympathetic nerves mediating sympathoinhibition (Harvey *et al.*, 1985; 1986; Willems *et al.*, 1985; Lokhandwala and Hegde, 1990; Missale *et al.*, 1998).

Regarding cardiac sympathoinhibition, several studies (e.g. Langer *et al.*, 1987; Lefevre-Borg *et al.*, 1987; Roquebert *et al.*, 1992) have shown that dopamine D_2-like receptors inhibit the cardioaccelerator sympathetic outflow in pithed rats since: (i) quinpirole (a D_2-like receptor agonist) inhibited the tachycardic responses to electrical stimulation of either the preganglionic sympathetic outflow or postganglionic cardioaccelerator nerves, but not those to i.v. isoprenaline or noradrenaline; and (ii) the antagonists sulpiride or domperidone (D_2-like), but not SCH 23390 (D_1-like) or idazoxan (α_2-adrenoceptor), blocked this response to quinpirole. However, to the best of our knowledge, no published study has thus far investigated the specific role of the D_2, D_3 and D_4 receptor subtypes mediating this response. Likewise, other groups have demonstrated the role of endogenous dopamine in modulating the cardioaccelerator sympathetic nerve activity in humans by domperidone-sensitive D_2-like receptors (Mannelli *et al.*, 1999; Lundby *et al.*, 2001). On this basis, the present study was designed to identify pharmacologically the specific D_2-like receptor subtypes (i.e. D_2, D_3 and D_4) involved in the above quinpirole-induced cardiac sympathoinhibition by analysing the effects of antagonists at the D_2 (L-741,626), D_3 (SB-277011-A) and D_4 (L-745,870) receptor subtypes (see Table 1), in doses high enough to block sympathoinhibitory D_2, D_3 and D_4 receptor subtypes in pithed rats (Ruiz-Salinas *et al.*, 2013).

Table 1

Receptor-binding affinities (pK_i) of the ligands used in the present study for cloned rat dopamine D_2, D_3 and D_4 receptor subtypes

	D_2	D_3	D_4
Agonist			
Quinpirole	8.3[a]	7.6[a]	7.5[a]
Antagonists			
L-741,626	7.9[b]	6.9[b]	6.1[b]
SB-277011-A	5.5[c]	8.0[c]	n.d.
L-745,870	5.7[d]	5.2[d]	8.8[d]

Data taken from: [a]Seeman and Van Tol (1994); [b]Bowery *et al.* (1996); [c]Reavill *et al.* (2000); [d]Patel *et al.* (1997).

Our results show that the D_2-like receptors inhibiting the cardioaccelerator sympathetic outflow resemble the pharmacological profile of the D_2 (but not of the D_3 or D_4) receptor subtype. These findings provide a basis for addressing the physiological relevance of this receptor and might help to open potential therapeutic avenues for the treatment of heart conditions related to the sympathoinhibitory role of the D_2 receptor subtype in humans. A preliminary account of this investigation was presented at the 2011 BPS Winter Meeting (Villalón *et al.*, 2011).

Methods

Animals

A total of 114 male Wistar normotensive rats (240–280 g) were used in the present experiments. The animals were maintained at a 12/12-h light-dark cycle (light beginning at 0700 h) and housed in a special room at constant temperature (22 ± 2°C) and humidity (50%), with food and water freely available in their home cages. All animal procedures and the protocols of the present investigation were approved by our Institutional Ethics Committee (CICUAL-Cinvestav) and followed the regulations established by the Mexican Official Norm (NOM-062-ZOO-1999), in accordance with the guide for the Care and Use of Laboratory Animals in the US and with the ARRIVE guidelines for reporting experiments involving animals (McGrath *et al.*, 2010).

General methods

After anaesthesia with diethyl ether and cannulation of the trachea, the rats were pithed by inserting a stainless steel rod through the orbit and *foramen magnum* into the vertebral

foramen (Shipley and Tilden, 1947). The animals were artificially ventilated with room air using an Ugo Basile pump (56 strokes min^{-1} and a stroke volume of 20 mL kg^{-1}; Ugo Basile Srl, Comerio, VA, Italy), as previously established (Kleinman and Radford, 1964). After cervical bilateral vagotomy, catheters were placed in: (i) the left and right femoral veins, for the infusions of agonists and i.v. bolus injections of antagonists, respectively; and (ii) the left carotid artery, connected to a Grass pressure transducer (P23XL, Grass Instrument Co., Quincy, MA, USA), for the recording of blood pressure. Heart rate was measured with a tachograph (7P4F, Grass Instrument Co.) triggered from the blood pressure signal. Both blood pressure and heart rate were recorded simultaneously by a model 7D Grass polygraph (Grass Instrument Co.). Then, the 114 rats were divided in two main sets, so that the responses to i.v. continuous infusions of either vehicle (i.e. physiological saline) or quinpirole could be investigated on the tachycardic responses induced by: (i) preganglionic (C_7-T_1) electrical stimulation of the cardioaccelerator sympathetic outflow (set 1; n = 102); or (ii) i.v. bolus injections of exogenous noradrenaline (set 2; n = 12). The tachycardic stimulus-response curves (S-R curves) and the dose-response curves (D-R curves) elicited by, respectively, preganglionic sympathetic stimulation and exogenous noradrenaline were completed in about 30 min, with no change in the baseline values of resting heart rate or blood pressure. The sympathetic tachycardic stimuli (0.03–3 Hz) as well as the i.v. dosing with noradrenaline (0.03–3 μg kg^{-1}) were given using a sequential schedule in 0.5 log unit increments, at 3–5 min intervals (see below). Each response was elicited under unaltered values of resting heart rate. The body temperature of each pithed rat was maintained at 37°C by a lamp and monitored with a rectal thermometer.

Experimental protocols

Protocol I. Electrical stimulation of the cardioaccelerator sympathetic outflow. In the first set of rats (n = 102), the pithing rod was replaced by an electrode enamelled except for 1 cm length 7 cm from the tip, so that the uncovered segment was situated at C_7-T_1 of the spinal cord to allow selective preganglionic stimulation of the cardioaccelerator sympathetic outflow, as previously reported (Sánchez-López *et al.*, 2003; 2004; Lozano-Cuenca *et al.*, 2009). A similar electrode was placed dorsally (Gillespie *et al.*, 1970). Before electrical stimulation, the animals received gallamine (25 mg kg^{-1}, i.v.) to avoid electrically induced muscular twitching. Since the cardiac sympathoinhibitory responses to several agonists in pithed rats are particularly more pronounced at lower frequencies of stimulation (Sánchez-López *et al.*, 2003; 2004), all the animals were systematically pretreated with 50 μg kg^{-1} (i.v.) of desipramine (a noradrenaline-reuptake inhibitor) 10 min before each S-R curve, as previously reported (Sánchez-López *et al.*, 2003; 2004). It should be pointed out that: (i) this dose of desipramine enhanced the tachycardic responses to sympathetic stimulation when compared to that in animals without desipramine (Villalón *et al.*, 1999); and (ii) the potentiating effect of desipramine on the sympathetically induced tachycardic responses did not wear off with time during the experiment. After a stable haemodynamic condition for at least 30 min, baseline values of diastolic blood pressure (a more accurate indicator of peripheral vascular resistance) and heart rate were determined. Then, the preganglionic cardiac sympathetic outflow was stimulated by applying trains of 10 s, consisting of monophasic rectangular pulses of 2 ms duration and 50 V, at increasing frequencies of stimulation (0.03, 0.1, 0.3, 1 and 3 Hz). When heart rate had returned to baseline levels, the next frequency was applied; this procedure was systematically performed until the S-R curve was completed (about 30 min). Subsequently, this set of animals was divided into three groups (n = 24, 30 and 48, respectively).

The first group (n = 24) was subdivided into four subgroups (n = 6 each) that received i.v. continuous infusions of: (i) saline (control; 0.02 mL min^{-1}, given twice); (ii) quinpirole (0.1 and 0.3 μg kg^{-1} min^{-1}); (iii) quinpirole (1 and 3 μg kg^{-1} min^{-1}); and (iv) quinpirole (3 and 10 μg kg^{-1} min^{-1}). Ten minutes after starting each infusion, a S-R curve was elicited again *during* the infusion of the corresponding compound. Once the S-R curve was completed, the infusion was stopped.

The second group (n = 30) was subdivided into five subgroups (n = 6 each) that received an i.v. bolus injection of, respectively: (i) bidistilled water (control; 1 mL kg^{-1}); (ii) 0.5% dimethyl sulphoxide (DMSO; 1 mL kg^{-1}); (iii) L-741,626 (300 μg kg^{-1}); (iv) SB-277011-A (300 μg kg^{-1}); and (v) L-745,870 (100 μg kg^{-1}). Ten minutes later, a S-R curve was elicited again, as described above, to analyse their effects *per se* on the sympathetically induced tachycardic responses.

The third group (n = 48) was subdivided into eight subgroups (n = 6 each) that received an i.v. bolus injection of, respectively: (i) bidistilled water (1 mL kg^{-1}); (ii) 0.5% DMSO (1 mL kg^{-1}); (iii) L-741,626 (100 μg kg^{-1}); (iv) L-741,626 (300 μg kg^{-1}); (v) SB-277011-A (100 μg kg^{-1}); (vi) SB-277011-A (300 μg kg^{-1}); (vii) L-745,870 (30 μg kg^{-1}); and (viii) L-745,870 (100 μg kg^{-1}). Ten minutes later, all subgroups received an i.v. continuous infusion of quinpirole (3 μg kg^{-1} min^{-1}). After 10 min, a S-R curve was elicited again as described above during the infusion of quinpirole.

Protocol II. Intravenous administration of exogenous noradrenaline. The second set of rats (n = 12) was prepared as described above, but the pithing rod was left and the administration of both gallamine and desipramine was omitted. After a stable haemodynamic condition for 30 min, baseline values of diastolic blood pressure and heart rate were determined. Then, the tachycardic responses were elicited by giving i.v. bolus injections of exogenous noradrenaline (0.03, 0.1, 0.3, 1 and 3 μg kg^{-1}), as previously reported (Sánchez-López *et al.*, 2004). When heart rate returned to baseline levels, the next dose was applied; this procedure was systematically performed until the D-R curve was completed (about 30 min). Subsequently, this set of animals was divided into two groups (n = 6 each) that received i.v. continuous infusions of: (i) physiological saline (0.02 mL min^{-1}); or (ii) quinpirole (3 μg kg^{-1} min^{-1}). Ten minutes later, a D-R curve to noradrenaline was elicited again during the infusion of the above compounds.

Other procedures applying to protocols I and II

The doses of quinpirole and saline were infused at 0.02 mL min^{-1} by a WPI model sp100i pump (World Precision

Instruments Inc., Sarasota, FL, USA). The intervals between the different stimulation frequencies or noradrenaline doses depended on the duration of the tachycardic responses (5–10 min), as in each case we waited until heart rate had returned to baseline values.

Data presentation and statistical evaluation

All data in the text and figures are presented as the mean ± SEM. The peak changes in heart rate produced by either electrical sympathetic stimulation or exogenous noradrenaline in the saline- and quinpirole-infused animals were determined. The difference in the values of diastolic blood pressure and heart rate within one subgroup of animals before and during the continuous infusions of saline or quinpirole (at the doses mentioned above) were evaluated with paired Student's *t*-test. Moreover, the difference between the changes in heart rate within one subgroup of animals was evaluated with the Student-Newman-Keuls test, once a two-way repeated measures ANOVA (randomized block design) had revealed that the samples represented different populations (Steel and Torrie, 1980). Statistical significance was accepted at $P < 0.05$ (two-tailed).

Drugs

Apart from the anaesthetic (diethyl ether), the drugs used in the present study (all obtained from Sigma Chemical Co., St. Louis, MO, USA) were the following: desipramine hydrochloride, gallamine triethiodide, noradrenaline hydrochloride, (±)-quinpirole dihydrochloride, (±)-3-[4-(4-chlorophenyl)-4-hydroxypiperidinyl]methylindole (L-741,626), 3-[[4-(4-chlorophenyl) piperazin-1-yl]methyl]-1H-pyrrolo[2,3-b] pyridine hydrochloride (L-745,870) and {*trans*-*N*-[4-[2-(6-cyano-1,2,3,4-tetrahydroisoquinolin-2-yl)ethyl]cyclohexyl]-4-quinolininecarboxamide} hydrochloride (SB-277011-A). All compounds were dissolved in physiological saline, except: (i) L-745,870 and SB-277011-A, which were dissolved in bidistilled water; and (ii) L-741,626 which was dissolved in 0.5% (vv^{-1}) DMSO in saline. These vehicles had no effect on the baseline values of diastolic blood pressure or heart rate (not shown). Fresh solutions were prepared for each experiment. The doses mentioned in this text refer to the free base of substances, except in the case of desipramine, gallamine and noradrenaline where they refer to the corresponding salts.

Results

Systemic haemodynamic variables

The baseline values of diastolic blood pressure and heart rate in the 114 rats were 69 ± 2 mmHg and 284 ± 5 beats min^{-1} respectively. After the first i.v. bolus injection of desipramine, both haemodynamic variables transiently increased ($P < 0.05$) to 74 ± 2 mmHg and 299 ± 6 beats min^{-1}, and returned to baseline values after 10 min (i.e. 70 ± 2 mmHg and 292 ± 5 beats min^{-1}). The subsequent treatments with desipramine did not modify further ($P > 0.05$) the baseline values of these variables. Moreover, in the different subgroups of rats pretreated with desipramine, the baseline values of diastolic blood pressure and heart rate were not significantly modified ($P > 0.05$; not shown) by: (i) the i.v. continuous infusions of saline or quinpirole; or (ii) the i.v. bolus injections of bidistilled water, 0.5% DMSO, L-741,626, SB-277011-A or L-745,870.

Initial effects produced by either electrical stimulation of the cardioaccelerator sympathetic outflow or i.v. noradrenaline on heart rate and blood pressure

The onset of the responses induced by stimulation (0.03–3 Hz) of the preganglionic (C_7-T_1) cardioaccelerator sympathetic outflow or i.v. bolus injections of exogenous noradrenaline (0.03–3 μg kg^{-1}) was immediate and resulted in frequency- or dose-dependent increases in heart rate (see below). In both cases, the tachycardic responses appeared about 10 s after starting each treatment and reached a maximum around 40 s after the treatment had ended. It is noteworthy that exogenous noradrenaline also produced dose-dependent increases in blood pressure (not shown), as previously described by Villalón *et al*. (1999); these vasopressor responses were not evaluated further. In all cases, the increases in heart rate elicited by electrical stimulation and exogenous noradrenaline were significant ($P < 0.05$) when compared with their corresponding baseline values. The electrically induced tachycardic responses were due to selective cardiostimulation since only negligible and inconsistent increases in blood pressure were observed, as previously reported (Villalón *et al*., 1999; Sánchez-López *et al*., 2003; 2004).

Effect of continuous infusions of saline or quinpirole on the tachycardic responses induced by either electrical sympathetic stimulation or exogenous noradrenaline

Figure 1 shows the tachycardic responses induced by electrical stimulation (S-R curves; upper panels) or exogenous noradrenaline (D-R curves; lower panels) before (control) and during i.v. infusions of: (i) saline (0.02 mL min^{-1} given twice for the S-R curves, or once for the D-R curves); or (ii) quinpirole (0.1–10 μg kg^{-1} min^{-1} for the S-R curves, or 3 μg kg^{-1} min^{-1} for the D-R curves). In the rats infused with saline, both the sympathetically induced (Figure 1A) and noradrenaline-induced (Figure 1E) tachycardic responses remained unchanged ($P > 0.05$). Besides, in the animals infused with quinpirole, the sympathetically induced tachycardic responses were: (i) unaltered ($P > 0.05$) during 0.1 and 0.3 μg kg^{-1} min^{-1} quinpirole (Figure 1B); and (ii) dose-dependently inhibited during 1 and 3 μg kg^{-1} min^{-1} quinpirole (Figure 1C) or 3 and 10 μg kg^{-1} min^{-1} quinpirole (with the resulting inhibition being significant at all stimulation frequencies vs. control; Figure 1D). The above inhibitions by quinpirole were unrelated to changes in baseline heart rate and diastolic blood pressure (not shown). Since the inhibition by 10 μg kg^{-1} min^{-1} quinpirole at most stimulation frequencies did not differ ($P > 0.05$) from that induced by 3 μg kg^{-1} min^{-1} quinpirole (except at 1 and 3 Hz) (Figure 1D), the inhibition by 3 μg kg^{-1} min^{-1} quinpirole (significant at all stimulation frequencies vs. control) was chosen for further pharmacological analysis in the rest of experiments (see below). In this respect, 3 μg kg^{-1} min^{-1} quinpirole did not

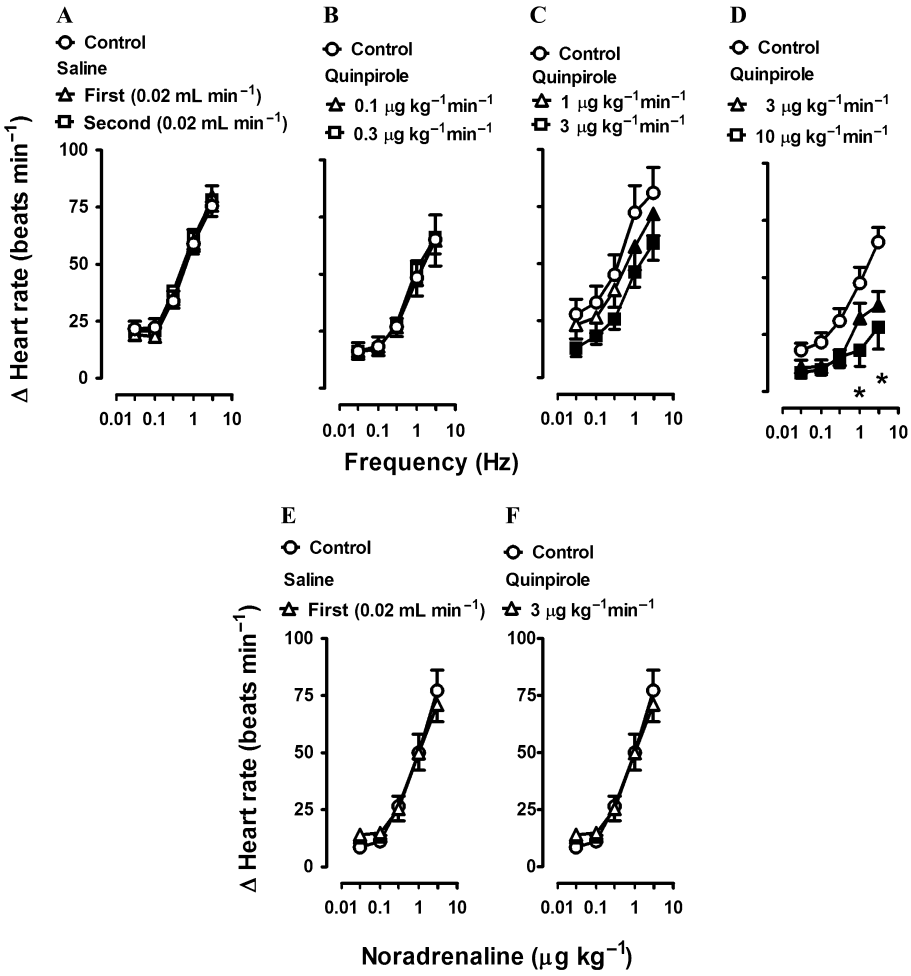

Figure 1

Increases in heart rate produced by electrical stimulation of the cardiac sympathetic outflow [stimulus-response (S-R) curves; *upper panels*] or i.v. bolus injections of exogenous noradrenaline [dose-response (D-R) curves; *lower panels*] before (control responses) and during i.v. continuous infusions of: (A,E) saline; or (B,C,D,F) quinpirole (n = 6 each). For the sake of clarity, empty symbols depict either control responses (○) or non-significant ($P > 0.05$) responses (△□) versus control; whereas solid symbols (▲■) represent significantly different responses ($P < 0.05$) versus control. *, $P < 0.05$ versus 3 μg kg^{-1} min^{-1} quinpirole. Δ Heart rate stands for 'increase in heart rate'.

modify ($P > 0.05$) the tachycardic responses to i.v. bolus injections of exogenous noradrenaline (see Figure 1F).

Effect of vehicles or antagonists at the D_2, D_3 and D_4 receptor subtypes per se *on the tachycardic responses produced by electrical sympathetic stimulation*

Figure 2 shows the electrically induced tachycardic responses before (control S-R curves) and after i.v. treatment with bidistilled water (1 mL kg^{-1}), 0.5% DMSO (1 mL kg^{-1}), L-741,626 (300 μg kg^{-1}), SB-277011-A (300 μg kg^{-1}) or L-745,870 (100 μg kg^{-1}). Clearly, the sympathetically induced tachycardic responses remained without significant changes after i.v. administration of bidistilled water (Figure 2A), 0.5% DMSO (Figure 2B), L-741,626 (Figure 2C), SB-277011-A (Figure 2D) or L-745,870 (Figure 2E). Likewise, the baseline values of diastolic blood pressure and heart rate were not significantly changed ($P > 0.05$) after administration of these compounds (not shown).

Effect of vehicles or antagonists at the D_2, D_3 and D_4 receptor subtypes on the quinpirole-induced inhibition of tachycardic responses produced by cardiac sympathetic stimulation

Figure 3 illustrates the sympathetically induced tachycardic responses before (control S-R curves) and after i.v. treatment with bidistilled water (1 mL kg^{-1}), 0.5% DMSO (1 mL kg^{-1}), L-741,626 (100 and 300 μg kg^{-1}), SB-277011-A (100 and 300 μg kg^{-1}) or L-745,870 (30 and 100 μg kg^{-1}) followed by 3 μg kg^{-1} min^{-1} quinpirole. The inhibition induced by quinpirole was: (i) unchanged by bidistilled water (Figure 3A) or 0.5% DMSO (Figure 3B); (ii) markedly blocked and abolished by, respectively, 100 and 300 μg kg^{-1} of the D_2 preferring

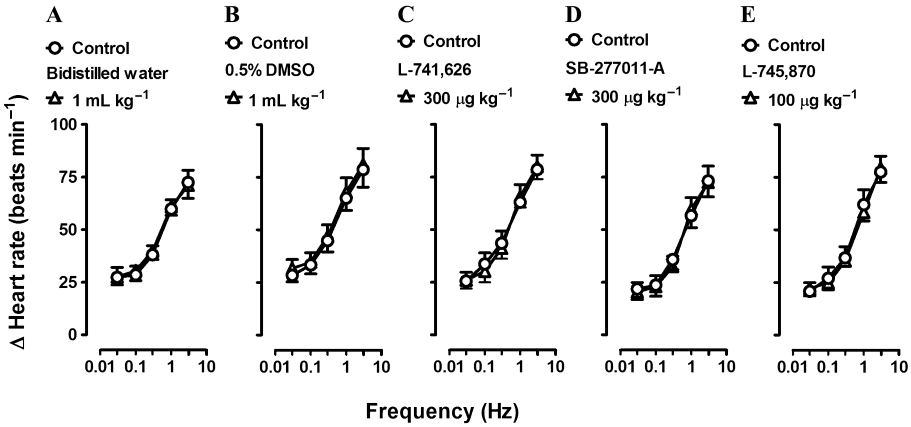

Figure 2

Effect *per se* of i.v. bolus injections of: (A) bidistilled water (1 mL kg^{-1}); (B) 0.5% DMSO (1 mL kg^{-1}); (C) L-741,626 (300 μg kg^{-1}); (D) SB-277011-A (300 μg kg^{-1}); or (E) L-745,870 (100 μg kg^{-1}) (n = 6 each) on the increases in heart rate produced by stimulation of the cardiac sympathetic outflow. Note that there were no significant differences (P > 0.05) in the S-R curves obtained before (control responses) and after administration of the different compounds. Δ Heart rate stands for 'increase in heart rate'.

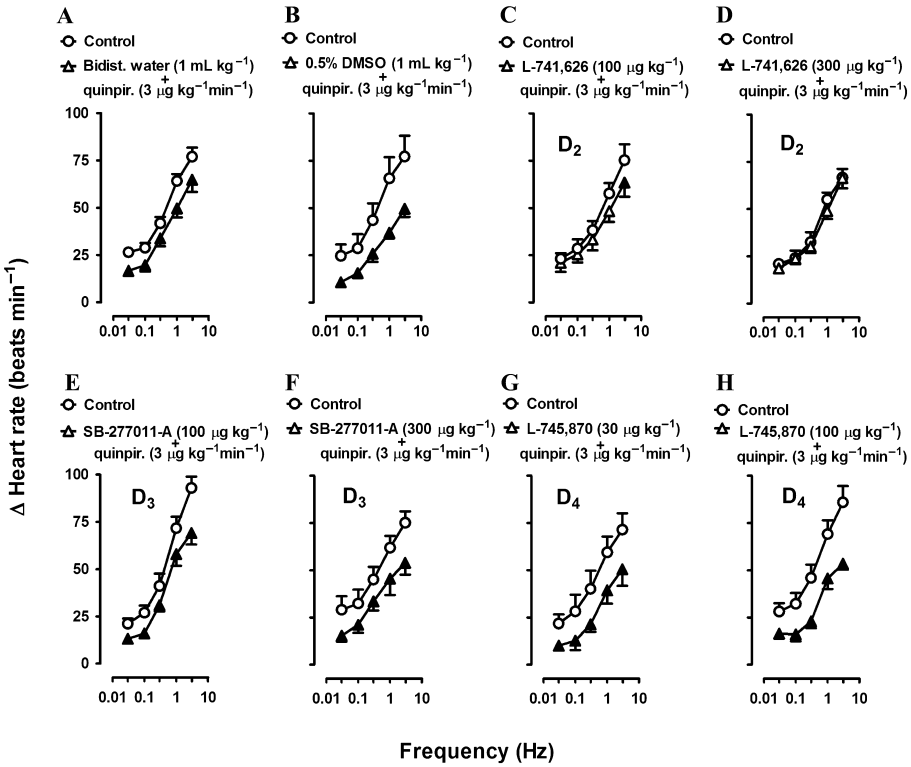

Figure 3

Effect of i.v. bolus injections of: (A) Bidistilled water (Bidist. water; 1 mL kg^{-1}); (B) 0.5% DMSO (1 mL kg^{-1}); (C) L-741,626 (100 μg kg^{-1}); (D) L-741,626 (300 μg kg^{-1}); (E) SB-277011-A (100 μg kg^{-1}); (F) SB-277011-A (300 μg kg^{-1}); (G) L-745,870 (30 μg kg^{-1}); or (H) L-745,870 (100 μg kg^{-1}) (n = 6 each) on the inhibition of sympathetically induced tachycardic responses induced by quinpirole (quinpir.; 3 μg kg^{-1} min^{-1}). The above compounds were injected after concluding the control S-R curve, 10 min before starting the infusion of quinpirole. For the sake of clarity, empty symbols depict either control responses (○) or non-significant (P > 0.05) responses (△) versus control; whereas solid symbols (▲) represent significantly different responses (P < 0.05) versus control. Δ Heart rate stands for 'increase in heart rate'.

receptor subtype antagonist L-741,626 (Figure 3C,D); and (iii) resistant to blockade by 100 and 300 μg kg^{-1} SB-277011-A (Figure 3E,F) or 30 and 100 μg kg^{-1} L-745,870 (Figure 3G,H).

Discussion and conclusions

General

Our study shows that the D_2-like receptors inhibiting the cardioaccelerator sympathetic outflow closely resemble the D_2 receptor subtype. Hence, quinpirole-induced cardiac sympathoinhibition was: (i) blocked by the D_2 receptor antagonist L-741,626; and (ii) resistant to blockade by the antagonists SB-277011-A (D_3) or L-745,870 (D_4). Moreover, the cardioaccelerator sympathetic nerve activity was not measured directly, but the electrically induced neurotransmitter release was estimated indirectly by assessing the evoked tachycardic response. Under these conditions, the responses to quinpirole were considered sympathoinhibitory since it inhibited the tachycardic responses to sympathetic stimulation without affecting those to i.v. noradrenaline (Figure 1).

Our findings agree with other results obtained from: (i) light microscopy autoradiography in male Wistar rat hearts, where the D_2 subtype was mainly located on catecholaminergic nerves (Cavallotti *et al.*, 2002); and (ii) $D_2^{-/-}$ knockout mice, which (as compared to wild-type mice) had higher adrenaline excretion, heart rate and blood pressure due to an increased sympathetic tone (Li *et al.*, 2001). Likewise, although not directly related with our findings, clinical studies using D_2-like receptor antagonists have shown increased values of: (i) sympathetic activity in healthy volunteers after domperidone (Mannering *et al.*, 1984); and (ii) heart rate, blood pressure and plasma catecholamines in patients with cardiovascular pathologies after metoclopramide (Bessa *et al.*, 1984; Kuchel *et al.*, 1985).

Systemic haemodynamic changes

The fact that diastolic blood pressure and heart rate were transiently increased after desipramine (see Results) can be attributed to an inhibition of noradrenaline-reuptake mechanisms (Bechtel *et al.*, 1986). Moreover, the potentiation of the sympathetically induced tachycardic responses by low stimulation frequencies after desipramine (for comparison see Villalón *et al.*, 1999) allowed us to reveal a more pronounced sympathoinhibition by quinpirole. Alternatively, the proposed sympathoinhibition by quinpirole may have been due to tachyphylaxis of the sympathetically induced tachycardic responses. However, this is unlikely since such responses remained unchanged during the saline infusions (Figure 1A) or the i.v. injections of vehicles (Figure 2A,B); consequently, no time-dependent changes occurred during our experimental protocols.

Since L-741,626, SB-277011-A or L-745,870 were devoid of any effect *per se* on the sympathetically induced tachycardic responses (Figure 2) or on the baseline diastolic blood pressure and heart rate (not shown), their doses were appropriate for investigating the receptors involved in quinpirole-induced sympathoinhibition (implying a direct interaction with its respective receptors on the cardioaccelerator nerve and/or autonomic ganglia; see below).

The cardiac sympathoinhibition induced by quinpirole: pharmacological correlation with the dopamine D_2 (rather than the D_3 or D_4) receptor subtype

For elucidating the specific role of D_2, D_3 and D_4 receptor subtypes in the cardiac sympathoinhibition by quinpirole, the antagonists L-741,626 (D_2 preferring), SB-277011-A (D_3) and L-745,870 (D_4) were used in doses that, considering their affinities (Table 1), were high enough to block sympathoinhibitory D_2, D_3 and D_4 subtypes in pithed rats (Ruiz-Salinas *et al.*, 2013). Although the possible interference by pharmacokinetic factors in pithed rats cannot be excluded, our findings suggest that the D_2-like receptors inhibiting the cardioaccelerator sympathetic outflow resemble the D_2 subtype, as this response was: (i) resistant to blockade by 100 and 300 μg kg^{-1} SB-277011-A (Figure 3E,F) or 30 and 100 μg kg^{-1} L-745,870 (Figure 3G,H); and (ii) markedly blocked and abolished by, respectively, 100 and 300 μg kg^{-1} L-741,626 (Figure 3C,D). Admittedly, the free plasma concentrations of these antagonists were not determined in our experimental model.

Nonetheless, the binding properties (Table 1) and profile of blockade (Figure 3C,D) displayed by L-741,626 deserve further considerations since it has a high affinity for the D_2 subtype, and a moderate affinity for the D_3 and D_4 subtypes. Hence, the *in vitro* D_2- versus D_3/D_4-selectivity of L-741,626 would seem small, leaving little room for *in vivo* selectivity. However, the lack of blockade by SB-277011-A and L-745,870 exclude the role of the D_3 and D_4 subtypes. Alternatively, it could be argued that the doses of these compounds were not enough to block their respective receptors and/or to reach the target tissues (i.e. cardioaccelerator nerve and/or sympathetic ganglia). Nevertheless, Ruiz-Salinas *et al.* (2013) showed in pithed rats that the same highest doses of SB-277011-A and L-745,870 abolished and weakly blocked, respectively, quinpirole-induced inhibition of the vasopressor sympathetic outflow.

Additional findings in support of cardiac sympathoinhibitory D_2 receptor subtypes

Other studies also reinforce the role of the D_2 receptor subtype mediating inhibition of the cardioaccelerator sympathetic outflow. For example: (i) Polakowski *et al.* (2004) reported that the subtype-selective D_2 receptor agonist PNU-95666E, but not BP897 (D_3) or PD168077 (D_4), decreased heart rate in anaesthetized rats; and (ii) Li *et al.* (2001) showed that D_2 receptor subtype knockout mice (as compared to wild-type mice) had a greater adrenaline excretion, a higher heart rate and hypertension (mainly due to an increased sympathetic tone). However, these experimental models cannot discriminate between peripheral and central effects.

It is noteworthy that L-741,626, SB-277011-A and L-745,870 (Figure 2C–E) failed to potentiate the electrically induced cardioaccelerator responses, probably because the rats were systematically pretreated with desipramine before each S-R curve. In any case, this finding: (i) implies that activation of D_2 (and also D_3 and D_4) receptor subtypes does not play an important role under physiological conditions; and (ii) does not exclude the role of the D_2 receptor subtype

under other conditions such as strenuous exercise. Indeed, several studies in humans have shown that intense exercise causes activation of sympathoinhibitory D_2-like receptors (which include the D_2, D_3 and D_4 subtypes), resulting in a decrease in circulating levels of noradrenaline (Mannelli *et al.*, 1999; Lundby *et al.*, 2001).

Transductional properties and possible locus of the sympathoinhibitory D_2 receptor subtypes

D_2-like (i.e. D_2, D_3 and D_4) receptor signalling is mediated by the heterotrimeric $G_{i/o}$ proteins that, among other effects, inhibit adenylyl cyclase activity, inactivate Ca^{2+} channels and/or activate inwardly rectifying K^+ channels (Neve *et al.*, 2004; Beaulieu and Gainetdinov, 2011). These are signal transduction systems usually associated with sympathoinhibition (Boehm and Kubista, 2002; De Jong and Verhage, 2009). Although our study provides no direct evidence that any of the above signalling mechanisms is involved, one might speculate upon the possible *locus* of the sympathoinhibitory D_2 receptor subtypes in our experimental model. In this respect, central mechanisms are not operative since pithed rats were used, but we cannot exclude an inhibitory action of quinpirole at both the autonomic ganglia and postganglionic sympathetic neurons (see Introduction section), which have modulatory D_2-like receptors (Wilffert *et al.*, 1984; Willems *et al.*, 1985). Moreover, the presence of D_2 (but not D_3 or D_4) receptor subtype mRNA has been shown in rat sympathetic neurons (Sigala *et al.*, 2000). Admittedly, further studies are required to ascertain the functional role of the D_2, D_3 and D_4 receptor subtypes on rat autonomic ganglia.

Final considerations on the differential effects of L-741,626, SB-277011-A and L-745,870 on quinpirole-induced inhibition of the cardioaccelerator and vasopressor sympathetic outflows

Overall, our results suggest that quinpirole-induced inhibition of the cardioaccelerator sympathetic outflow is mainly mediated by the dopamine D_2 receptor subtype, with no pharmacological evidence for the role of the D_3 and D_4 receptor subtypes. In contrast, Ruiz-Salinas *et al.* (2013) have shown, using the same compounds, same doses, same i.v. routes and same species as in our present investigation, that quinpirole-induced inhibition of the vasopressor sympathetic outflow resembles the pharmacological profile of the D_3 receptor subtype and, to a lesser extent, of the D_4 receptor subtype, with no evidence for the role of the D_2 subtype. Both studies, taken together, represent an interesting case where, depending on whether the vasopressor or cardioaccelerator sympathetic outflow is measured, the same doses of the antagonists L-741,626 (D_2 preferring), SB-277011 (D_3) and L-745,870 (D_4) (which correlate with their corresponding pK_i values; Table 1) will have opposite effects on quinpirole-induced sympathoinhibition. Admittedly, we have no clear-cut explanation but, probably, the anatomical origin of these sympathetic outflows might account for such a difference. As shown by Gillespie *et al.* (1970), the spinal (preganglionic) origin of the vasopressor sympathetic outflow (T_7-T_9) differs from that of the cardioaccelerator sympathetic outflow (C_7-T_1). Interestingly, a difference in pharmacological profile has also been shown for the vasopressor and cardioaccelerator sympathoinhibition induced by 5-hydroxytryptamine (5-HT) in male Wistar rats, namely: (i) 5-HT_{1A}, 5-HT_{1B} and 5-HT_{1D} receptors inhibit the vasopressor sympathetic outflow (Villalón *et al.*, 1998); and (ii) 5-HT_{1B}, 5-HT_{1D} and 5-$HT_{5A/5B}$ receptors inhibit the cardioaccelerator sympathetic outflow (Sánchez-López *et al.*, 2003; 2004). Evidently, further multidisciplinary research will be required to ascertain the possible physiological relevance of these differences.

Lastly, it is tempting to suggest (although we have no direct experimental evidence) that the doses of the above antagonists were high enough to reach the target tissues (i.e. cardioaccelerator nerve and/or sympathetic ganglia). Accordingly, 300 μg kg^{-1} of L-741,626 and L-745,870 attenuated *per se* the sympathetically induced vasopressor responses (Ruiz-Salinas *et al.*, 2013).

In conclusion, the cardiac sympathoinhibition induced by 3 μg kg^{-1} min^{-1} quinpirole in pithed rats mainly resembles the pharmacological profile of the dopamine D_2 receptor subtype, with no pharmacological evidence for the involvement of the D_3 or D_4 subtypes. This provides new findings for understanding the modulation of the cardioaccelerator sympathetic outflow.

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Conflict of interest

The authors state no conflict of interest.

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