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Short communication

In vivo evidence that isoproterenol may increase heart rate in the rat by mechanisms in addition to activation of cardiac β_1- or β_2-adrenoceptors

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Abstract

This study demonstrates that the tachycardia produced by systemic injections of the β-adrenoceptor agonist, isoproterenol (10 μg/kg, i.v.), in conscious rats were not reduced after injection of the selective β_1-adrenoceptor antagonist, atenolol (1 mg/kg, i.v.), or after subsequent injection of the $\beta_{1,2}$-adrenoceptor antagonist, propranolol (1 mg/kg, i.v.). The hypotensive responses produced by isoproterenol were slightly diminished by atenolol and markedly diminished by propranolol. The tachycardia produced by catecholamines released for cardiac sympathetic nerve terminals were blocked by atenolol. These results suggest that the hypotensive actions of a 10 μg/kg dose of isoproterenol are mediated by activation of $\beta_{1,2}$-adrenoceptors whereas the increases in heart rate may be due to activation of another type of β-adrenoceptor in cardiac pacemaker cells.

Keywords: β-adrenoceptor; Catecholamine; Isoproterenol; Heart rate; Pacemaker cell

1. Introduction

The endogenous catecholamines, norepinephrine and epinephrine, exert their effects by activation of multiple adrenoceptors including β_1-, β_2- and β_3-adrenoceptors and atypical β-adrenoceptors (Blue et al., 1989; Emorine et al., 1994; Cohen et al., 1995a,b, 1999; Kaumann, 1996; Kaumann and Molenaar, 1996; Malinkowski and Schlicker, 1996). Catecholamines are potent agonists of β_1- and β_2-adrenoceptors but weaker agonists at β_3-adrenoceptors whereas isoproterenol (*N*-alkylated-norepinephrine) is a potent agonist of all three sub-types of β-adrenoceptors (see Emorine et al., 1994). Moreover, the β_3-adrenoceptor is not blocked by doses of propranolol which block β_1- and β_2-adrenoceptors (see Cohen et al., 1999).

Systemic injections of hypotensive agents elicit baroreceptor reflex-mediated increases in heart rate due to an increase in sympathetic nerve activity and the withdrawal of cardiovagal drive. The sympathetic component of the baroreceptor reflex-mediated increases in heart rate are virtually eliminated by the β_1-adrenoceptor antagonist, atenolol (Head and McCarty, 1987). This suggests that the tachycardia produced by catecholamines released from cardiac sympathetic nerve terminals is mediated primarily by activation of β_1-adrenoceptors on cardiac pacemaker cells. The systemic injection of isoproterenol produces falls in mean arterial blood pressure which are accompanied by increases in heart rate (Whalen et al., 1999a,b). It could be expected that this tachycardia involves baroreceptor reflex-mediated changes in autonomic nerve activity and the direct effects of isoproterenol on cardiac β-adrenoceptors. The main aim of this study was to determine whether the increases in heart rate produced by systemic injections of isoproterenol in conscious rats involve the activation of β_3-adrenoceptors and/or atypical β-adrenoceptors as well as $\beta_{1,2}$-adrenoceptors in cardiac pacemaker cells.

2. Materials and methods

2.1. Rats and surgical procedures

The protocols were approved by the University of Iowa Animal Care and Use Committee. Sprague–Dawley rats (250–350 g) were anesthetized with pentobarbital (50 mg/kg, i.v.) and a catheter was inserted into a femoral vein to give drugs and in the lower abdominal aorta via a femoral artery to measure pulsatile and mean arterial pressure and to determine heart rate (see Whalen et al.,

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1999a,b). The catheters were exteriorized at the back of the neck and all wounds were closed. The rats were allowed 3–4 days to recover from surgery.

2.2. Experimental procedures

Rats ($n = 8$) received injections of sodium nitroprusside (10 μg/kg, i.v.) and isoproterenol (1 and 10 μg/kg, i.v.) before and between 10 and 20 min after injection of saline and again between 10 and 20 min after another injection of saline. The injections of saline were given 30 min apart. Other rats ($n = 8$) received injections of sodium nitroprusside (10 μg/kg, i.v.) and isoproterenol (1 and 10 μg/kg, i.v.) before and between 10 and 20 min after injection of atenolol (1 mg/kg, i.v.) and again after injection of propranolol (1 mg/kg, i.v.). The injections of atenolol and propranolol were given 30 min apart. Other rats ($n = 6$) received injections of sodium nitroprusside (10 μg/kg, i.v.) and isoproterenol (1 and 10 μg/kg, i.v.) before and between 20 and 40 min after combined injection of the angiotensin AT1 receptor antagonist, losartan (10 mg/kg, i.v.) (Wong et al., 1990), the muscarinic receptor antagonist, methyl-atropine (1 mg/kg, i.v.), and propranolol (2.5 mg/kg, i.v.). The effects of each injection of sodium nitroprusside and isoproterenol were allowed to subside completely before another injection was given. All drugs were dissolved and diluted for injection in saline (0.9% NaCl w/v). The data are presented as mean ± S.E.M. and were analyzed by repeated measures analysis of variance followed by modified Student's *t*-test with Bonferroni corrections for multiple comparisons. A value of $P < 0.05$ was taken to denote statistical significance.

3. Results

3.1. Effects of saline, atenolol and propranolol on resting cardiovascular parameters

The effects of saline, atenolol and propranolol on resting parameters are summarized in Table 1. The two injections of saline did not affect mean arterial blood pressure or heart rate. The administration of atenolol (1 mg/kg, i.v.) did not affect mean arterial blood pressure but produced an immediate and sustained fall in heart rate. The subsequent administration of propranolol (1 mg/kg, i.v.) did not affect mean arterial blood pressure and did not further lower heart rate.

Table 1
Effects of atenolol and the subsequent administration of propranolol on resting mean arterial blood pressures and heart rates of conscious rats
Each value represents the mean ± S.E.M. MAP = mean arterial blood pressure. HR = heart rate. n = number of rats. Saline_{I} and saline_{II} = injection 1 and injection 2 of saline. ATN = atenolol (1 mg/kg, i.v.). PROP = propranolol (1 mg/kg, i.v.).

Group	n	Phase	MAP (mm Hg)	HR (bpm)
I	8	Pre	116 ± 2	358 ± 8
		Post-saline_{I}	115 ± 3	364 ± 9
		Post-saline_{II}	114 ± 2	357 ± 9
II	8	Pre	112 ± 3	367 ± 10
		Post-atenolol	109 ± 3	318 ± 12[a]
		Post-propranolol	108 ± 3	317 ± 9[a]

[a] $P < 0.05$, post-treatment vs. pre.

Table 2
Effects of atenolol and the subsequent administration of propranolol on the cardiovascular effects of sodium nitroprusside and isoproterenol in conscious rats
Each value represents the mean ± S.E.M. MAP = mean arterial blood pressure. HR = heart rate. SNP = sodium nitroprusside. ISO = isoproterenol. ATN = atenolol (1 mg/kg, i.v.). PROP = propranolol (1 mg/kg, i.v.). There were five rats in each group.

Agent	Dose (μg/kg, i.v.)	Parameter	Pre	Post-ATN	Post-PROP
SNP	10	ΔMAP (mm Hg)	−17 ± 2	−20 ± 3	−19 ± 3
		ΔHR (bpm)	+93 ± 7	+34 ± 4[a]	+25 ± 4[a,b]
ISO	1	ΔMAP (mm Hg)	−19 ± 3	−17 ± 2	−3 ± 2[a]
		ΔHR (bpm)	+97 ± 6	+57 ± 7[a]	+49 ± 6[a]
ISO	10	ΔMAP (mm Hg)	−31 ± 2	−24 ± 2[a]	−11 ± 3[a,b]
		ΔHR (bpm)	+143 ± 7	+156 ± 8	+149 ± 10

[a] $P < 0.05$, post-atenolol or post-atenolol + propranolol vs. pre.
[b] $P < 0.05$, post-atenolol + propranolol vs. post-atenolol.

3.2. Effects of saline, atenolol and propranolol on the cardiovascular responses to sodium nitroprusside and isoproterenol

The effects of atenolol (1 mg/kg, i.v.) and propranolol (1 mg/kg, i.v.) on the responses produced by sodium nitroprusside (10 μg/kg, i.v.) and isoproterenol (1 and 10 μg/kg, i.v.) are summarized in Table 2. The sodium nitroprusside-induced depressor responses were not affected by atenolol or by propranolol. The increases in heart rate in response to the sodium nitroprusside-induced depressor responses were substantially attenuated by atenolol (−63 ± 6%, $P < 0.05$, post-atenolol vs. pre). The residual tachycardia was slightly diminished after subsequent administration of propranolol (−22 ± 5%, $P < 0.05$, post-atenolol + propranolol vs. post-atenolol).

The 1 μg/kg dose of isoproterenol produced a fall in mean arterial blood pressure and an increase in heart rate. These responses were similar in magnitude to those produced by sodium nitroprusside ($P > 0.05$, for both comparisons). The isoproterenol-induced depressor response was not affected by atenolol but was abolished after administration of propranolol. The isoproterenol-induced tachycardia was substantially attenuated by atenolol. The residual tachycardia was not diminished after the subsequent administration of propranolol. The 10 μg/kg dose of isoproterenol produced a fall in mean arterial blood pressure and an increase in heart rate. These responses were greater than those produced by the 1 μg/kg dose of

isoproterenol ($P < 0.05$, for both comparisons). The isoproterenol-induced depressor response was slightly diminished by atenolol ($-19 \pm 4\%$, $P < 0.05$, post-atenolol vs. pre). The residual depressor response was diminished after subsequent injection of propranolol ($-56 \pm 7\%$, $P < 0.05$, post-atenolol + propranolol vs. post-atenolol). The increases in heart rate produced by the 10 μg/kg dose of isoproterenol were not attenuated by atenolol or after subsequent administration of propranolol. The duration of the tachycardia produced by the 1 μg/kg dose of isoproterenol (196 ± 23 s) was substantially diminished after administration of atenolol (72 ± 8 s, $-59 \pm 7\%$ of pre-atenolol values, $P < 0.05$). The duration of the tachycardia was not further attenuated after subsequent injection of propranolol (64 ± 10 s, $-7 \pm 6\%$ of post-atenolol values, $P > 0.05$). The duration of the tachycardia produced by the 10 μg/kg dose of isoproterenol (378 ± 46 s) was slightly diminished after administration of atenolol (326 ± 26 s, $-12 \pm 3\%$ of pre-atenolol values, $P < 0.05$) but was not further diminished after subsequent injection of propranolol (317 ± 28 s, $-2 \pm 7\%$ of post-atenolol values, $P > 0.05$). The cardiovascular effects produced by the above doses of sodium nitroprusside and isoproterenol were similar before and after each injection of saline ($P > 0.05$, for all comparisons, data not shown).

3.3.Effects of combined treatment with losartan, methyl-atropine and propranolol on the cardiovascular responses to sodium nitroprusside and isoproterenol

Resting mean arterial blood pressure values before and 15–20 min after combined injection of losartan (10 mg/kg, i.v.), methyl-atropine (1 mg/kg, i.v.) and propranolol (2.5 mg/kg, i.v.) were 110 ± 2 and 108 ± 3 mm Hg, respectively ($-1 \pm 4\%$, $P > 0.05$). Resting heart rate values before and 15–20 min after administration of the treatments were 364 ± 10 and 342 ± 12 bpm, respectively ($-6 \pm 1\%$, $P < 0.05$). The depressor response produced by sodium nitroprusside (10 μg/kg, i.v.) was slightly greater after than before administration of the treatments (-21 ± 3 vs. -16 ± 2 mm Hg, $+31 \pm 5\%$, $P < 0.05$). However, the increases in heart rate associated with these depressor responses were completely abolished after administration of the treatments. The increases in heart rate before and after administration of the treatments were $+86 \pm 7$ and $+4 \pm 3$ bpm, respectively ($P < 0.05$). The depressor response produced by the 1 μg/kg dose of isoproterenol was abolished after administration of the treatments (pre vs. post, -18 ± 3 vs. -3 ± 2 mm Hg, $-89 \pm 7\%$, $P < 0.05$). The increase in heart rate produced by the 1 μg/kg dose of isoproterenol was markedly smaller but not abolished after administration of the treatments (pre vs. post, $+93 \pm 10$ vs. $+24 \pm 4$ bpm, $-72 \pm 8\%$, $P < 0.05$). The depressor response produced by the 10 μg/kg dose of isoproterenol was markedly reduced but not abolished after administration of the treatments (pre vs. post, -33 ± 3 vs. -11 ± 2 mm Hg, $-68 \pm 6\%$, $P < 0.05$). The increase in heart rate produced by the 10 μg/kg dose of isoproterenol was similar before and after administration of the treatments (pre vs. post, $+137 \pm 10$ vs. $+128 \pm 12$ bpm, $-6 \pm 5\%$, $P < 0.05$). The duration of the tachycardia produced by the 1 μg/kg dose of isoproterenol was markedly reduced after administration of the treatments ($-63 \pm 7\%$, $P < 0.05$). The duration of the tachycardia produced by the 10 μg/kg dose of isoproterenol was slightly reduced after administration of the treatments ($-14 \pm 3\%$, $P < 0.05$).

4. Discussion

The depressor responses produced by systemic injections of sodium nitroprusside were associated with pronounced increases in heart rate. These increases in heart rate were markedly attenuated by the β_1-adrenoceptor antagonist, atenolol. The residual tachycardia was slightly attenuated after the subsequent administration $\beta_{1,2}$-adrenoceptor antagonist, propranolol. These increases in heart rate are due to baroreceptor reflex mediated increases in cardiac sympathetic nerve activity and the withdrawal of cardiovagal drive (Head and McCarty, 1987). Accordingly, the residual tachycardia observed after blockade of $\beta_{1,2}$-adrenoceptors is probably due to baroreflex-mediated withdrawal of cardiovagal tone since sodium nitroprusside-induced tachycardia is virtually abolished by a combination of atenolol and the muscarinic receptor antagonist, methyl-atropine (Head and McCarty, 1987). Taken together, these findings suggests that the increases in heart rate produced by catecholamines released from sympathetic nerve terminals are mediated primarily by activation of β_1-adrenoceptors and to a lesser extent by activation of β_2-adrenoceptors.

The depressor response produced by the 1 μg/kg dose of isoproterenol was not affected by atenolol but was substantially diminished by the subsequent administration of propranolol. The depressor responses produced by the 10 μg/kg dose of isoproterenol was slightly diminished by atenolol and substantially diminished by propranolol. The isoproterenol-induced falls in mean arterial blood pressure are due to reductions in peripheral vascular resistances (Whalen et al., 1999a,b). Taken together, it appears that isoproterenol dilates resistance arteries in vivo primarily by activation of β_2-adrenoceptors in these vessels although the higher dose of isoproterenol appears to activate functionally important β_1-adrenoceptors. The observation that the combined administration of atenolol and propranolol did not eliminate the hypotensive response produced by the 10 μg/kg dose of isoproterenol suggests that this dose was able to dilate resistance vessels by activation of β-adrenoceptors other than β_1- or β_2-adrenoceptors.

The increase in heart rate produced by the 1 μg/kg dose of isoproterenol was substantially but not completely

attenuated by atenolol. The duration of this tachycardia was also substantially attenuated by atenolol. The subsequent administration of propranolol did not affect the residual tachycardia. It appears that this dose of isoproterenol increases heart rate by activation of β_1-adrenoceptors and by β-adrenoceptors which are resistant to atenolol and propranolol. The increase in heart rate produced by the 10 μg/kg dose of isoproterenol was not affected by atenolol or by the subsequent administration of propranolol. In addition, the duration of the tachycardia was only slightly diminished after administration of atenolol. This shows that this higher dose of isoproterenol elicits a full tachycardia despite blockade of β_1- and β_2-adrenoceptors. The combined administration of methyl-atropine and losartan and a high dose of propranolol (2.5 mg/kg, i.v.), did not affect the increases in heart rate produced by the 10 μg/kg dose of isoproterenol. This suggests that the tachycardia produced by the high dose of isoproterenol is not mainly due to changes in vagal activity or the release of renin from the kidney. Taken together, these findings support the possibility that the atenolol/propranolol-insensitive tachycardia produced by isoproterenol is due to direct actions on cardiac pacemaker cells. Whether these actions are due to activation of cardiac β_3-adrenoceptors remains to be determined.

There is an increasing amount of evidence to suggest that β_3-adrenoceptors and atypical β-adrenoceptors are expressed in a variety of human and animal tissues (Blue et al., 1989; Emorine et al., 1994; Cohen et al., 1995a,b; Malinkowski and Schlicker, 1996) including cardiac cells (Kaumann, 1996; Kaumann and Molenaar, 1996; Cohen et al., 1999). Although the physiological roles of β_3-adrenoceptors and atypical β-adrenoceptors have yet to be established (see Emorine et al., 1994), the present findings raise the possibility that higher doses of isoproterenol may exert their effects, in part, by activation of these receptors. Peroxynitrite markedly attenuates the vasodilator actions of isoproterenol in the hindquarter bed of pentobarbital-anesthetized rats whereas it does not affect the vasodilator actions of isoproterenol in the mesenteric bed of these rats (Benkusky et al., 1999). Peroxynitrite is a potent oxidant and readily nitrates free and protein-associated tyrosine residues (see Benkusky et al., 1998, 1999). β_1-, β_2- and β_3-adrenoceptors possess extracellular cysteine residues which may be susceptible to oxidation by peroxynitrite (Probst et al., 1992; Emorine et al., 1994). In addition, β_1- and β_2-adrenoceptors possess extracellular tyrosine residues susceptible to peroxynitrite-induced nitration whereas β_3-adrenoceptors do not (Probst et al., 1992; Emorine et al., 1994). It is possible that β_3-adrenoceptor exists in the mesenteric bed and, that unlike β_1- and β_2-adrenoceptors, this β-adrenoceptor is resistant to nitration by peroxynitrite. The β_3-adrenoceptor in cardiac and vascular tissue may represent a 'fail-safe' β-adrenoceptor which allows sympathetic and circulating catecholamines, and exogenous β-adrenoceptor agonists, to exert cardiovascular effects under conditions which favor the excessive formation of peroxynitrite (see Benkusky et al., 1998, 1999).

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