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Short communication

Blockade of β-adrenoceptors enhances cAMP signal transduction in vivo

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Abstract

The aim of this study was to determine whether the blockade of β-adrenoceptors would enhance cAMP-mediated signal transduction processes in vivo. The administration of the membrane permeable cAMP analogue, 8-(4-chlorophenylthiol)-cAMP (8-CPT-cAMP, 10 μmol/kg, i.v.) produced an increase in heart rate ($+27 \pm 2\%$, $P < 0.05$), a fall in mean arterial blood pressure ($-21 \pm 3\%$, $P < 0.05$) and falls in hindquarter ($-12 \pm 3\%$, $P < 0.05$) and mesenteric ($-32 \pm 3\%$, $P < 0.05$) vascular resistances in pentobarbital-anesthetized rats. The β-adrenoceptor antagonist, propranolol (1 mg/kg, i.v.) lowered heart rate ($-12 \pm 3\%$, $P < 0.05$) but did not affect mean arterial blood pressure or vascular resistances. The tachycardia, hypotension and vasodilation produced by 8-CPT-cAMP were exaggerated after administration of propranolol ($P < 0.05$ for all comparisons). The nitric oxide-donor, sodium nitroprusside (2 μg/kg, i.v.), produced falls in mean arterial blood pressure and vascular resistances of similar magnitude to those produced by 8-CPT-cAMP. These sodium nitroprusside-induced responses were unaffected by propranolol ($P < 0.05$ for all comparisons). Sodium nitroprusside also produced a minor increase in heart rate ($+5 \pm 1\%$, $P < 0.05$) which was abolished by propranolol. These findings suggest that 8-CPT-cAMP directly increases heart rate and that blockade of β-adrenoceptors enhances the potency of cAMP within the heart and vasculature. © 1998 Elsevier Science B.V. All rights reserved.

Keywords: β-Adrenoceptor; Heart; Vasculature; Nitric oxide (NO); In vivo

1. Introduction

Norepinephrine released from cardiac sympathetic nerves increases heart rate by activation of cardiac G_s protein-coupled β_1-adrenoceptors (Harden, 1983). Activation of these receptors stimulates adenylate cyclase which converts ATP to cAMP. Intracellular cAMP exerts its effects on cardiac function by activation of cAMP-dependent protein kinase (Koch et al., 1995). Chronic infusions of β_1-adrenoceptor agonists down-regulate cardiac β_1-adrenoceptors and attenuate β-adrenoceptor-mediated increases in heart rate and cardiac contractility (Kenakin and Ferris, 1983; Marsh et al., 1980; Vatner et al., 1989). However, it is not known whether the loss of receptors totally accounts for the tachyphylaxis to β-adrenoceptor agonists. This is important because agonists can produce maximal responses when there has been a substantial reduction in the affinity and/or density of receptors (Harden, 1983).

The vasodilator effects of β-adrenoceptor agonists are mediated principally by activation of G_s protein-coupled β_2-adrenoceptors in vascular smooth muscle (Harden, 1983). The chronic administration of β-adrenoceptor agonists results in the down-regulation of β_2-adrenoceptors in the vasculature and tachyphylaxis to the vasodilator effects of these agonists (Harden, 1983; Stein et al., 1995). However, there is also evidence that tachyphylaxis to β-adrenoceptor agonists may involve the down-regulation of cAMP-mediated vasorelaxant processes in vascular smooth muscle due to the enhanced degradation of cAMP by phosphodiesterases (Bourne et al., 1973) and the enhanced expulsion of cAMP from cells (Brunton and Mayer, 1979). It is also possible that tachyphylaxis to β-adrenoreceptor agonists may involve an impaired cAMP-mediated activation of cAMP-dependent protein kinase or the diminished

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capacity of activated cAMP-dependent protein kinase to modulate vascular tone (Harden, 1983).

These findings raise the possibility that a change in β-adrenoreceptor function may affect cAMP signal transduction processes. The aim of this study was to determine whether blockade of β-adrenoceptors may alter the potency of intracellular cAMP. The blockade of β-adrenoceptors with propranolol may mimic the situation of agonist-induced down-regulation of β-adrenoceptors. If this is the case, then it may be expected that the potency of cAMP would be diminished after administration of propranolol. As such, we determined whether the hemodynamic effects of the membrane-permeable cAMP analogue, 8-(4-chlorophenylthiol)-cAMP (8-CPT-cAMP), would be exaggerated after administration of the β-adrenoceptor antagonist, propranolol.

2. Materials and methods

2.1. Rats and surgical procedures

The protocols described below were approved by the University of Iowa Animal Care and Use Committee. Sprague–Dawley rats (250–350 g, $n = 21$) were anesthetized with pentobarbital (50 mg/kg, i.p.) and polyethylene catheters were placed into the femoral vein for drug administration and in the lower abdominal aorta via the femoral artery for direct measurement of pulsatile and mean arterial blood pressure. A midline laparotomy was performed and pulsed Doppler flow probes were placed around the superior mesenteric arteries and the lower abdominal aorta to measure blood flow velocities to determine mesenteric and hindquarter vascular resistances, as described previously (Kooy and Lewis, 1996). During surgery and experimentation, animal body temperature was maintained at 37°C via a thermostat controlled heating pad. The rats were allowed to breathe room air supplemented with carboxygen (95% O_2–5% CO_2) via a face mask. The arterial catheter was connected to a Beckman Dynograph-coupled pressure transducer (Cobe Laboratories) for the measurement of pulsatile arterial blood pressure and mean arterial blood pressure. Heart rate was determined from the pulsatile arterial pressure by way of a Beckman Dynograph-coupled cardiotachometer.

2.2. Experimental protocols

We determined the hemodynamic effects produced by 8-CPT-cAMP (10 μmol/kg, i.v.) and isoproterenol (10 μg/kg, i.v.) before and 15–20 min after administration of either saline (0.9% NaCl w/v, i.v; $n = 5$) or propranolol (1 mg/kg, i.v; $n = 5$). The propranolol-induced decreases in heart rate had reached their plateau values at this time (see Section 3). We also determined the hemodynamic effects produced by the nitric oxide (NO)-donor, sodium nitroprusside (2 μg/kg, i.v.), before and 15–20 min after administration of either saline (0.9% NaCl w/v, i.v; $n = 5$) or propranolol (1 mg/kg, i.v; $n = 6$).

2.3. Drugs

Sodium pentobarbital and sodium nitroprusside were obtained from Abbott Laboratories (North Chicago, IL, USA). Propranolol, isoproterenol and 8-CPT-cAMP were obtained from Sigma (St. Louis, MO, USA).

2.4. Statistical analyses

The data are presented as the mean ± S.E.M. of the maximal changes in the hemodynamic parameters produced by all test agents. The data were analyzed by repeated measures analysis of variance followed by Student's modified *t*-test with the Bonferroni correction for multiple comparisons between means (Kooy and Lewis, 1996).

3. Results

3.1. Effects of propranolol on resting hemodynamic parameters

The effects of propranolol (1 mg/kg, i.v.) on resting hemodynamic parameters of pentobarbital-anesthetized rats ($n = 11$) are summarized in Table 1. Propranolol reduced heart rate ($P < 0.05$) but did not affect mean arterial blood pressure or hindquarter or mesenteric vascular resistances. The administration of saline (0.9% NaCl w/v, i.v.) did not affect the resting hemodynamic parameters of pentobarbital-anesthetized rats ($P > 0.05$ for all comparisons, data not shown).

3.2. Effects of propranolol on the hemodynamic effects of isoproterenol

The injection of isoproterenol (10 μg/kg, i.v.) in saline-treated rats ($n = 5$) produced pronounced falls in

Table 1
The effects of propranolol on resting hemodynamic parameters

Parameter	Pre-	Post-propranolol	% Change
HR (bpm)	335 ± 8	296 ± 8	−12 ± 3[a]
MAP (mmHg)	121 ± 3	116 ± 4	−4 ± 2
HQR (mmHg/kHz)	43 ± 3	47 ± 4	+9 ± 6
MR (mmHg/kHz)	28 ± 4	32 ± 3	+14 ± 9

HR = heart rate; MAP = mean arterial blood pressure; HQR = hindquarter resistance; MR = mesenteric resistance.
The dose of propranolol was 1 mg/kg, i.v.
Each value represents the mean ± S.E.M. the actual data or the percent changes in these parameters.
There were 11 rats in the group.
[a] $P < 0.05$, significant response.

mean arterial blood pressure ($-52 \pm 6\%$, $P < 0.05$) and hindquarter ($-60 \pm 7\%$, $P < 0.05$) and mesenteric ($-54 \pm 5\%$, $P < 0.05$) vascular resistances, and a substantial increase in heart rate ($+26 \pm 3\%$, $P < 0.05$). This dose of isoproterenol did not affect any of these parameters in propranolol (1 mg/kg, i.v; $n = 5$)-treated rats ($P > 0.05$ for all comparisons, data not shown).

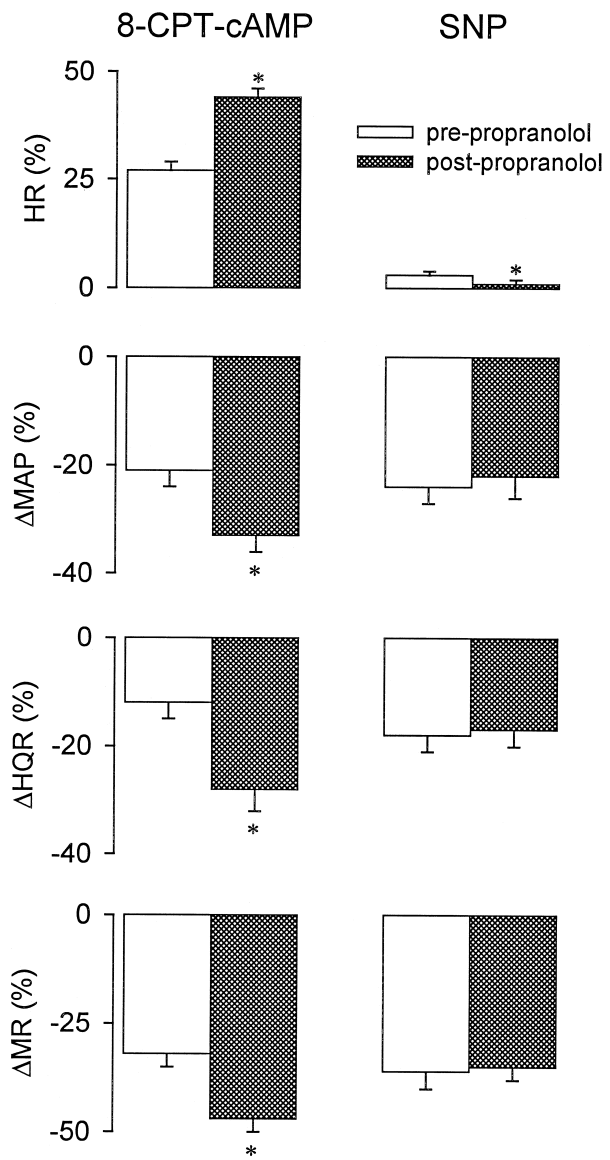

Fig. 1. A summary of the maximal effects of 8-(4-chlorophenylthiol)-cAMP (8-CPT-cAMP, 10 μmol/kg, i.v.; $n = 5$) or sodium nitroprusside (SNP, 2 μg/kg, i.v.; $n = 5$) on heart rate (HR), mean arterial blood pressure (MAP) and hindquarter (HQR) and mesenteric (MR) vascular resistances of pentobarbital-anesthetized rats before and after administration of propranolol (1 mg/kg, i.v.). The data are expressed mean ± S.E.M. of the percent changes in these parameters. * $P < 0.05$, post-propranolol vs. pre.

3.3. Effects of propranolol on the hemodynamic effects of 8-CPT-cAMP

The percent changes in hemodynamic parameters produced by 8-CPT-cAMP (10 μmol/kg, i.v.) in pentobarbital-anesthetized rats ($n = 5$) before and after administration of propranolol (1 mg/kg, i.v.) are summarized in Fig. 1. 8-CPT-cAMP produced a fall in mean arterial blood pressure and in hindquarter and mesenteric vascular resistances and a substantial increase in heart rate ($P < 0.05$ for all responses). These 8-CPT-induced responses were exaggerated after the administration of propranolol ($P < 0.05$ for all post-propranolol vs. pre-responses). Since propranolol did not affect resting MAP or vascular resistances, the arithmetic changes in these values produced by 8-CPT-cAMP would be greater after than before the administration of propranolol. Although propranolol lowered resting heart rate, the arithmetic changes produced by 8-CPT-cAMP were larger after than before administration of propranolol. The arithmetic changes in heart rate before and after administration of propranolol were, $+85 \pm 5$ and $+108 \pm 6$ bpm, respectively (arithmetic difference between the responses of $+23 \pm 5$ bpm, $P < 0.05$). The hemodynamic effects of 8-CPT-cAMP were similar before and after the administration of saline ($P > 0.05$ for all comparisons, data not shown). In addition, the hemodynamic effects of 8-CPT-cAMP after the administration of propranolol were greater than those after the administration of saline ($P < 0.05$ for all comparisons).

3.4. Effects of propranolol on the hemodynamic effects of sodium nitroprusside

The hemodynamic effects produced by sodium nitroprusside (2 μg/kg, i.v.) in pentobarbital-anesthetized rats ($n = 5$) before and after administration of propranolol (1 mg/kg, i.v.) are also summarized in Fig. 1. Sodium nitroprusside lowered mean arterial blood pressure and hindquarter and mesenteric vascular resistances and produced a minor increase in heart rate ($P < 0.05$ for all responses). The hypotensive and vasodilator effects of sodium nitroprusside were unaffected by propranolol ($P > 0.05$ for all post-propranolol vs. pre-responses) whereas the tachycardia was abolished ($P < 0.05$). The hemodynamic effects of sodium nitroprusside were similar before and after administration of saline ($P > 0.05$ for all comparisons, data not shown).

4. Discussion

The rationale for the present study was that blockade of β-adrenoceptors may alter the biological potency of cAMP in the heart and vasculature. The systemic injection of the membrane-permeable cAMP analogue, 8-CPT-cAMP (10 μmol/kg, i.v.), produced a fall in mean arterial blood

pressure associated with vasodilation in the hindquarter and mesenteric beds. The vasodilator effects 8-CPT-cAMP support existing evidence that G_s protein-coupled receptor agonists regulate vascular resistance via the generation of cAMP (Harden, 1983). For example, the systemic injection of the G_s protein-coupled receptors agonists, isoproterenol, and pituitary adenylate cyclase activating polypeptide-27, produce pronounced vasodilator responses in the hindquarter and mesenteric beds of anesthetized rats (Travis et al., 1997). The 8-CPT-cAMP-mediated fall in vascular resistance was greater in the mesenteric than in the hindquarter bed. At present, we do not know whether the enhanced 8-CPT-cAMP-mediated vasodilation in the mesenteric bed is because cAMP-dependent vasodilation is more pronounced in this bed or that 8-CPT-cAMP is degraded less efficiently than in the hindquarter bed. Propranolol did not affect the hypotension or vasodilation produced by sodium nitroprusside. The vasodilator actions of sodium nitroprusside are attributed to its decomposition to NO which relaxes vascular smooth muscle via the generation of cGMP although this compound may have other mechanisms of action (Stamler et al., 1992). Nonetheless, our results suggest that the blockade of β-adrenoceptors may not affect NO-mediated cGMP signaling in vascular smooth muscle. A principal finding of this study was that the hypotensive and vasodilator effects of 8-CPT-cAMP were exaggerated after the administration of propranolol. This suggests that the blockade of β-adrenoceptors enhances cAMP signaling in resistance vessels. The mechanisms by which blockade of β-adrenoceptors enhances cAMP activity may include, (i) diminished degradation of cAMP by phosphodiesterases, (ii) diminished expulsion of cAMP from cells, (iii) enhanced ability of cAMP to activate cAMP-dependent protein kinase, or (iv) enhanced ability of cAMP-dependent protein kinase to modulate intracellular processes regulating vascular tone. It is also possible that propranolol interferes with the degradation of 8-CPT-cAMP in the bloodstream or accentuates its entry into vascular smooth muscle. Although the results with propranolol suggest that the blockade of β-adrenoceptors up-regulates cAMP signaling in vascular smooth muscle, it will be necessary to examine the effects of other β-adrenoceptor antagonists to ensure that this hypothesis is correct.

The fall in mean arterial blood pressure produced by isoproterenol was accompanied by a pronounced tachycardia which was abolished by the β-adrenoceptor antagonist, propranolol. In contrast, the fall in mean arterial blood pressure produced by the NO-donor, sodium nitroprusside, was associated with minimal changes in heart rate. The systemic injection of this dose of sodium nitroprusside produces a pronounced baroreceptor-reflex-mediated tachycardia in conscious rats (Davisson et al., 1996). This suggests that pentobarbital anesthesia prevents baroreceptor-mediated increases in sympathetic drive which would occur in response to falls in mean arterial blood pressure. These results also suggest that the tachycardia produced by isoproterenol in pentobarbital-anesthetized rats is due to the direct activation of cardiac β-adrenoceptors rather than baroreceptor-mediated increases in sympathetic drive. The fall in mean arterial blood pressure produced by 8-CPT-cAMP was also accompanied by a pronounced tachycardia. We have obtained preliminary evidence that the tachycardia produced by 8-CPT-cAMP in pentobarbital-anesthetized rats are not affected by ganglionic blockade (Whalen et al., unpublished observations). Since the 8-CPT-cAMP-induced increases in heart rate are not attenuated by propranolol (see below), it is likely that the tachycardic effect of 8-CPT-cAMP is due to the entry of this membrane-permeable cAMP analogue into the pacemaker cells of the heart. This is further supported by evidence that the systemic injection of cAMP (10 μmol/kg, i.v.) does not affect heart rate ($-2 \pm 1\%$, $n = 5$; $P > 0.05$) in pentobarbital-anesthetized rats.

The other principal finding was that the tachycardia produced by 8-CPT-cAMP was actually exaggerated in the presence of propranolol. This suggests that the blockade of cardiac β-adrenoceptors (Harden, 1983) increases the ability of cAMP to regulate heart rate. The exaggerated potency of intracellular cAMP to regulate heart rate may be due to the mechanisms described above or may involve alterations in NO synthesis. β-Adrenoceptor agonists increase pacemaker rate by alteration of ion-channel activity including the cAMP-mediated activation of voltage-sensitive L-type Ca^{2+}-channels (Ca^{2+}_{VSL}-channels) (Han et al., 1994). The increase in intracellular Ca^{2+} resulting from activation of Ca^{2+}_{VSL}-channels would stimulate NO synthase activity in cardiac tissues (Han et al., 1994; Schulz et al., 1992). NO generated within isolated cardiac pacemaker cells markedly diminishes the chronotropic effects of β-adrenoceptor agonists via increases in cGMP (Hare et al., 1995). Moreover, there is considerable evidence that intracellular cGMP counteracts the effects of cAMP in the heart (Hare et al., 1995). As such, it is possible that the blockade of β-adrenoceptors may indirectly diminish NO synthesis by reducing the influx of Ca^{2+} into cardiac pacemaker cells. The resultant loss of cGMP would lead to the up-regulation of cAMP signaling.

It is also likely that propranolol will abolish β-adrenoceptor-mediated activation of cAMP-dependent protein kinase and G protein-coupled receptor kinases in vascular and cardiac tissue (Hausdorff et al., 1990; Palczewski and Benovic, 1991; Premont et al., 1995). These protein kinases are known to cause the homologous desensitization of β-adrenoceptors and the heterologous desensitization of other G_s protein-coupled receptors (Harden, 1983; Palczewski and Benovic, 1991; Hausdorff et al., 1990). As such, the blockade of β-adrenoceptors may increase the potency of other G_s protein-coupled receptor agonists by increasing the potency of cAMP signaling and by reducing the heterologous desensitization of these G_s protein-coupled receptors.

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