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Role of the postsynaptic α_2-adrenergic receptor subtypes in catecholamine-induced vasoconstriction

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Abstract

Catecholamines induce direct vasoconstriction mediated by postsynaptic α-adrenergic receptors (α-ARs) of both the α_1 and α_2 type. To evaluate the contribution of each α_2-AR subtype (α_{2A}, α_{2B}, and α_{2C}) to this function, we used groups of genetically engineered mice deficient for the gene to each one of these subtypes and compared their blood pressure (BP) responses to their wild-type counterparts. Blood pressure responses to a bolus of norepinephrine (NE) were assessed before and after sequential blockade of α_1-ARs with prazosin and α_2-ARs with yohimbine. The first NE bolus elicited a brief 32 to 44 mm Hg BP rise ($p < 0.001$ from baseline) in all six groups. Prazosin decreased BP by 23 to 33 mm Hg in all groups, establishing a new lower baseline. Repeat NE at that point elicited lesser but still significant ($p < 0.001$) brief pressor responses between 32% and 45% of the previous BP rise in five of the six groups. Only the α_{2A}-AR gene knockouts differed, responding instead with a 20-mm Hg fall in BP, a significant change from baseline ($p < 0.001$) and different from the pressor response of their wild-type counterparts ($p < 0.001$). The addition of yohimbine produced no further BP change in the five groups, but it did produce a small 7.5-mm Hg fall ($p < 0.05$) in the α_{2A}-AR knockouts. Norepinephrine bolus during concurrent α_1 and α_2-AR blockade produced significant ($p < 0.001$) hypotensive responses in all subgroups, presumably attributable to unopposed stimulation of β_2-vascular wall ARs. We conclude that the α_2-AR-mediated vasoconstriction induced by catecholamines is attributable to the α_{2A}-AR subtype because mice deficient in any one of the other subtypes retained the capacity for normal vasoconstrictive responses. However, the α_1-ARs account for the major part (as much as 68%) of catecholamine-induced vasoconstriction. © 2000 Elsevier Science Inc. All rights reserved.

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1. Introduction

α_2-Adrenergic receptors (α_2-ARs) are believed to play an important role in mediating the sympathetic nervous system (SNS) effects on blood pressure (BP). Three subtypes of α_2-AR, designated as α_{2A}, α_{2B}, and α_{2C}, were first suspected pharmacologically several years ago (Murphy and Bylund, 1988) and were subsequently confirmed by molecular biology techniques (Bylund, 1992). They are activated to a variable extent by catecholamines, exerting different effects depending of their localization. Because of a lack of subtype-selective pharmacological compounds and radioligands, efforts to elucidate the exact function(s) of each α_2-AR subtype have had limited success.

Recently, the creation of genetically engineered mice deficient in each one of the α_2-AR subtypes (Altman, et al., 1999; Link et al., 1996, 1995) became a valuable tool in dissecting the physiological effects of catecholamines mediated by each subtype. Evidence from studies using such animals has shown that the α_{2A}-AR subtype located in the central nervous system (CNS) and concentrated in the brainstem (Tavares et al., 1996), which is known to be the center of cardiovascular control, is responsible for the tonic regulation of the SNS (MacDonald et al., 1997; MacMillan et al., 1996; Makaritsis et al., 1999a). The α_{2B}-AR, thought to be the only one located in the vascular smooth muscle cells of the arterial wall, was proposed as having a role in the peripheral vasoconstrictor action (Altman et al., 1999; Link et al, 1996; MacMillan et al., 1996). Recent data from our laboratory showed that the α_{2B}-AR subtype is indeed necessary in the hypertensive response to salt loading,

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but no conclusion could be drawn from those studies about whether this role is central or peripheral (Makaritsis[b] et al., 1999b). No hemodynamic responses mediated by the α_{2C}-AR are known so far (Link et al., 1996).

The purpose of the current experiments was to evaluate the contribution of each α_2-AR subtype in α_2-AR-mediated peripheral vasoconstriction. To this aim, we studied the pressor effects of direct α_2-AR stimulation by the administration of norepinephrine (NE) in animals deficient for the α_{2A}-AR (−/−), α_{2B}-AR (+/−), or α_{2C}-AR(−/−) gene compared with their wild-type controls. Because the pressor effects of NE that are mediated by the postsynaptic α_1-AR are quantitatively much more important in peripheral vasoconstriction than those mediated by the postsynatic α_2-AR (Makaritsis et al., 2000), we first blocked the α_1-AR with prazosin, so that any changes in blood pressure elicited by NE should be attributed to activation of the vascular α_2-AR.

2. Materials and methods

2.1. Animals

Six groups of male mice 7–11 weeks old and weighing 22–31 g were used in this study: one group of each homozygous (−/−) knockout mice for the α_{2A}-AR (n = 11), an α_{2C}-AR (n=10) subtype, one group of heterozygous (+/−) α_{2B}-AR subtype gene-deficient mice, and their their wild-type counterparts (n = 10 for each group). We used heterozygous α_{2B}-AR gene-deficient mice because homozygous α_{2B} (−/−) do not breed well to yield sufficient numbers. Heterozygous α_{2B}-AR gene knockout mice have proved to be acceptable for such studies (Makaritsis et al., 1999a, 1999b, 2000), because they have been shown to have a very low level of expression the α_{2B}-AR protein (Link et al., 1996).

Genotypes were determined by the polymerase chain reaction (PCR) from DNA isolated from the tail or spleen of the animals as described elsewhere (Makaritsis et al., 1999a, 1999b, 2000). In brief, to screen the α_{2A}-AR line, MA.GF1, MAGB1, and PGK.2 primers were used to detect the intact α_{2A}-AR gene (246 bp) or the interrupted α_{2A}-AR gene (368 bp). To screen the α_{2B}-AR lines, MB.GF2, MB.GB2, and PKG0.1 primers were used to detect the intact (365-bp) or interrupted (750-bp) α_{2B}-AR gene. Three others sets of primers (MC.GF1, MC.GB1, and PGK0.3) were used to detect the intact (377-bp) or interrupted (540-bp) α_{2C}-AR gene. The presence of the PGK.neoBpa insert was confirmed with the use of neo.F1 and neo.B3 primers to produce a 548-bp band by PCR. Each 25 μl PCR contained 0.2 μmol/l each primer, 0.2 mmol/l each dNTP, 2 mmol/l Mg^{2+}, 10 mmol/l Tris-HCl, pH 8.3, 50 mmol/l KCl, and 0.025 U AmpliTaq Gold (Perkin Elmer). After incubation, samples were loaded on 3–4% Nusieve Agarose (FMC)

All animals were kept under a 12-h light/dark cycle in the animal facility of our institution and given free access to food (Purina Certified Rodent Chow, 5002) and distilled water. All experiments were conducted in accordance with guidelines for the Care and Use of Animals approved by the Boston University Medical Center.

2.2. Surgical procedure

Surgery was performed under anesthesia with intraperitoneal sodium pentobarbital (50 mg/kg). A modified polyethylene catheter was introduced in the right iliac artery for BP recording, and a silastic tubing was placed in the right iliac vein for drug administration, as described elsewhere (Johns et al., 1996). After surgery, the animals were returned to their cages and allowed an overnight recovery period.

2.3. Experimental protocol

On the day after catheterization, the arterial line was connected to a BP transducer, and mean BP was recorded with a computerized data-acquisition system (Power Lab/400, AD Instruments Pty Ltd, Caste Hill, Australia). The venous line was connected to a Harvard infusion pump (Harvard Aparatus, Holliston, MA) for drug infusion. The baseline BP was recorded for at least 30 min or until it became stable. At this point, a 100-μl bolus of 1.2 μg/kg NE was injected, and BP changes were recorded. After BP had returned to baseline, a 100-μl bolus of prazosin (1.5 mg/kg) was injected, followed by a 1.5 mg/kg infusion. In previous experiments, we found this dose to completely block the response of α_1-AR to selective agonists (although it may have a weak affinity to α_2-AR as well).

When a new baseline was established for at least 30 min, NE (1.2 μg/kg) was injected again, and BP changes were recorded. Then a 100-μl bolus of the nonselective α_2-blocker yohimbine (2 mg/kg) was injected, and a 2-mg/kg infusion was started along with the continuing prazosin infusion to block all α_2-AR as well. At least 30 min later when BP was steady, NE was injected again, and ensuing changes in BP were recorded.

2.4. Drugs

The following drugs were used: norepinephrine (bitartrate salt, Sigma Chemical Co., St. Louis, MO); prazosin hydrochloride (Sigma Chemical Co., St. Louis, MO); ascorbic acid (Sigma Chemical Co., St. Louis, MO); and yohimbine hydrochloride (RBI, Natick, MA). All drugs were dissolved in 0.9% saline with the exception of prazosin, which was dissolved in 5% dextrose. Ascorbic acid (1 mg/ml) was added to the NE solution to prevent oxidation. Bolus injections were given in a volume of

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2.5. Statistical analysis

All values are expressed as mean ± SEM. Student's t-tests for paired and unpaired data were used as appropriate. The Mann-Whitney rank sum test was used for nonparametric data. Differences at $p < 0.05$ were considered significant.

3. Results

The effects of sequential drug administration on BP in each group of α_2-AR gene knockout mice and their wild-type counterparts are illustrated in Figure 1. Table 1 gives in detail the numbers corresponding to these changes in BP after each manipulation.

The first injection of NE elicited a significant ($p < 0.001$) hypertensive response in all animals. This hypertensive response to NE lasted only 1–2 min, after which BP returned to preinjection value. Then prazosin caused a significant fall in BP, which was similar in all groups of mice, and established a new baseline. Repeat administration of NE bolus produced a smaller, but still highly significant ($p < 0.001$), increase in BP in five of the six groups. In contrast with the other groups, the α_{2A}-AR knockouts responded to this maneuver with a fall in BP, which was significant in regard to both their own baseline ($p < 0.001$) and to the response of their wild-type counterparts ($p < 0.001$; Table 1).

The addition of a nonselective α_2-AR blockade by yohimbine while the α_1-AR blockade by prazosin continued did not alter the BP in five of the six groups; however, in the α_{2A}-AR knockouts, it further decreased the BP by an average 7.5 ± 3.1 mm Hg ($p = 0.034$), thus establishing a new baseline in that group. At that point, another bolus of NE caused a significant fall in BP ($p < 0.001$) from the new baseline in all six groups, ranging from 15.2 mm Hg to 28 mm Hg and lasting for a couple of minutes, with no differences between knockouts and wild-type groups.

4. Discussion

Circulating catecholamines induce vasoconstriction mediated by postsynaptic α-AR (Aburto et al., 1995; Chen et al., 1988; Timmermans et al., 1987; Young et al., 1988). It has long been known that this is predominantly an α_1-AR function, with a lesser contribution from the α_2-AR (Gavras et al., 1995). The purpose of the present experiments was to assess the contribution of each α_2-AR receptor subtype to the direct vasoconstricting effect elicited by bolus injections of norepinephrine. By using genetically altered mice deficient for the α_{2A}-AR, α_{2B}-AR, or α_{2C}-AR genes and sequentially blocking the α_1-AR and remaining α_2-AR with successive infusions of α_1- and α_2-blocking agents, we attempted to identify the α_2-AR subtype. The main finding of these experiments was that vasoconstriction mediated by direct activation of vascular α_2-ARs is attributable to the α_{2A}-AR subtype. Indeed, norepinephrine injected after α_1-AR blockade with prazosin (which has a 1000-fold greater affinity for α_1 than α_2-AR) elicited in all, except the α_{2A}-AR knockouts, a BP rise amounting to 32–45% of that produced by the same injection before prazosin, indicating that 55–68% of this hypertensive response was due to the α_1-AR. More importantly, the α_{2A}-AR gene knockouts under prazosin blockade responded to norepinephrine with a transient hypotensive reaction. This hypotensive response was similar to the one elicited subsequently by norepinephrine in all subgroups after pretreatment with both yohimbine and prazosin, to ensure concurrent blockade of all α_2-AR types.

The data indicate that as much as 68% of adrenergically induced vasoconstriction is mediated by peripheral postsynaptic α_1-AR, confirming previously existing knowledge (Gavras et al., 1995). The remainder would be due to stimulation of α_2-AR located on the vascular wall. Because both the α_{2B}-AR and the α_{2C}-AR gene-deficient mice exhibited the same degree of hypertensive response as did their wild-type counterparts, whereas the α_{2A}-AR gene knockouts were unable to raise their BP in response to NE, we had to conclude that the peripheral postsynaptic α_{2A}-AR is the α_2-AR subtype mediating vasoconstriction. Given the capacity of prazosin for partial α_{2B}-AR blockade in addition to α_1-AR blockade, the fact that NE elicited comparable pressor responses in α_{2B}-AR +/+ and α_{2B}-AR +/− mice would not be sufficient evidence that the α_{2B}-AR does not contribute to the pressor response. However, the fact remains that all five groups (except the α_{2A} −/−) exhibited vasoconstriction in response to NE under prazosin infusion. Indeed, only after the addition of yohimbine was all α_2-AR-mediated vasoconstriction completely abolished. These data indicate that the presence of the α_{2A}-AR but not the α_{2B}-AR subtype is necessary for this vasoconstrictive response to NE. This is consistent with our earlier studies, where only α_{2A}-AR but not α_{2B}-AR mRNA could be detected on the arterial wall of rabbits (Handy et al., 1998). This interpretation, however, is opposite that given by other authors (Link et al., 1996; MacDonald et al., 1997) of their data, for which they concluded that the α_{2B}-AR has a role in the peripheral vasoconstrictive effect elicited by adrenergic agonists. Nevertheless, their data are not in conflict with ours, because the pressor action attributed to α_{2B}-AR stimulation in their studies could in fact be attributable to CNS rather than peripheral α_{2B}-ARs. Indeed, our subsequent series of studies on salt-induced hypertension in mice deficient in each one of these α_2-AR subtypes (Makaritsis et al., 1999a, 1999b, 2000) led us to conclude that the central presynaptic α_{2B}-AR appears

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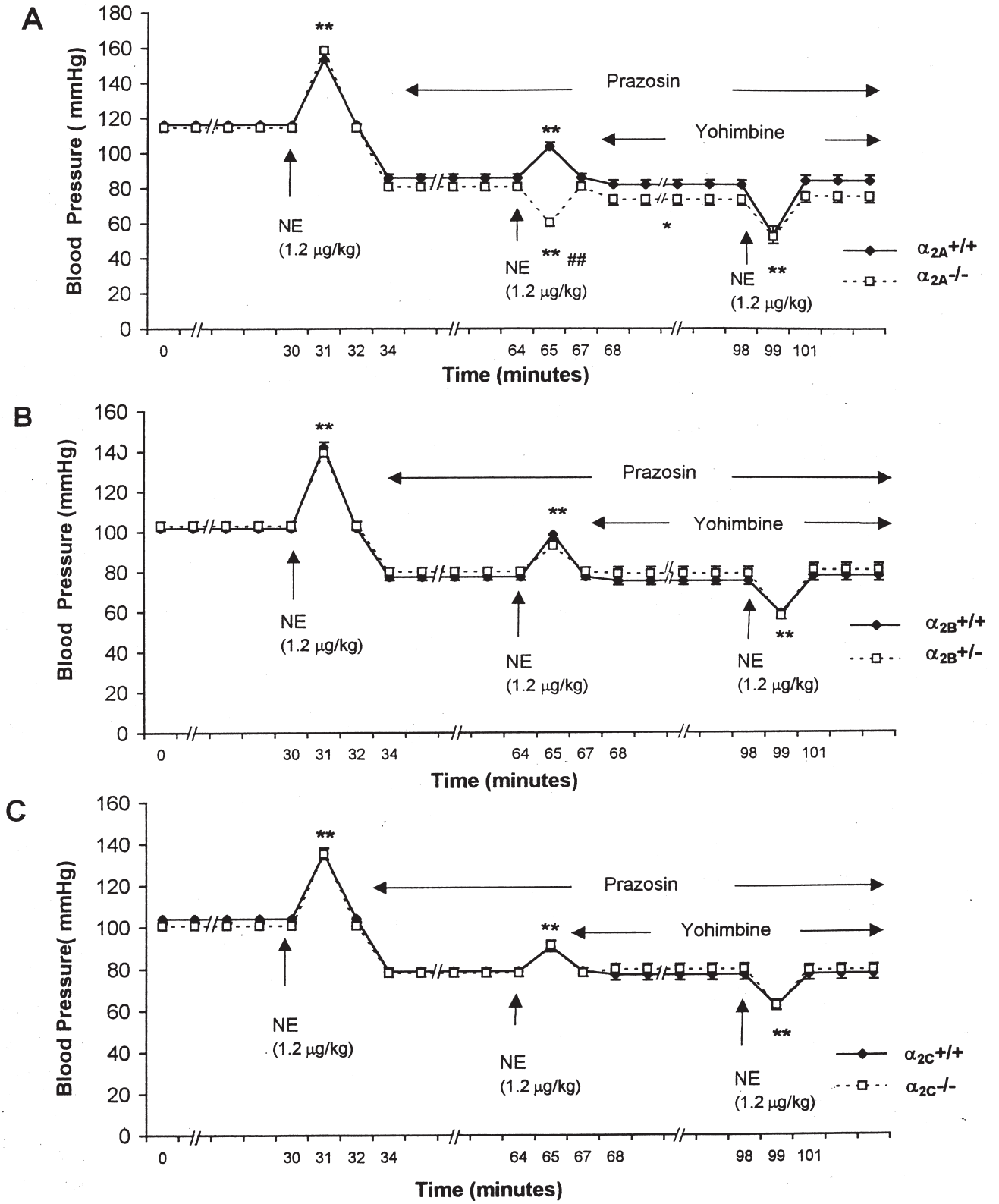

Fig. 1. Mean blood pressure responses to sequential IV administration of α_2-adrenergic agonists and antagonists in mice deficient for the gene of each α_2-AR subtype, compared with their wild-type counterparts. Key: NE, norepinephrine; ◆, wild type; □, α_2-AR subtype deficient. Values are expressed as mean ± SEM, symbols denote significant changes in blood pressure in each group of mice versus their own baseline (** $p < 0.001$, * $p < 0.05$) and in knockout animals versus their wild-type controls (## $p < 0.001$).

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Table 1
Effects of various manipulations on BP in six groups of animals

Group	Mean BP (mm Hg) Baseline	ΔBP (mm Hg) in response to each manipulation				
		After NE**	After α_1 blockade**	NE after α_1 blockade**	After $\alpha_1 + \alpha_2$ blockade	NE after $\alpha_1 + \alpha_2$ blockade**
α_{2A} (+/+) (n = 10)	116.2 ± 0.6	37.6 ± 2.77	−30.1 ± 2.02	17.8 ± 1.53	−3.9 ± 2.75	−28.6 ± 3.18
α_{2A} (−/−) (n = 11)	114.6 ± 1.4	44.2 ± 5.5	−33.6 ± 2	−20.5 ± 2#	−7.5 ± 2*	−20.7 ± 2.8
α_{2B} (+/+) (n = 10)	101.8 ± 2.1	40.3 ± 6.1	−24.4 ± 2	13.1 ± 1.9	2.1 ± 2.7	−16.1 ± 2.3
α_{2B} (+/−) (n = 15)	103.1 ± 1	36.4 ± 3.9	−23 ± 2	12.9 ± 1.9	−1.0 ± 1.2	−21.1 ± 2.5
α_{2C} (+/+) (n = 10)	104.1 ± 2	31.4 ± 2.54	−25.3 ± 2.2	11.6 ± 1.5	−1.8 ± 1.6	−15.2 ± 3.23
α_{2C} (−/−) (n = 10)	100.7 ± 2	34.4 ± 2.7	−22.8 ± 2.3	13.2 ± 1.6	1.7 ± 2.5	−17.4 ± 3.5

Note: Values are expressed as mean ± SEM. Changes in BP are compared with the baseline before each drug administration. # $p < 0.001$ between α_{2A} − AR knockout mice and their wild-type counterparts. * $p < 0.05$ for α_{2A} −/− mice versus their previous baseline. ** $p < 0.001$ from baseline for all values in column.

the central α_{2A}-AR (which is the predominant presynaptic α_2-AR in the CNS) is indeed responsible for the hypotensive sympathoinhibitory effects attributed to presynaptic α_2-AR stimulation, as concluded by these investigators (Altman et al., 1999; MacDonald et al., 1997; MacMillan et al., 1996) and by our own studies (Makaritsis et al., 1999a, 2000).

It is possible that different postsynaptic α_2-AR subtypes might mediate direct vasoconstriction to different extents in various vascular beds (Phillips, et al., 1997; Ping and Faber, 1993). It is notable in this respect that, in the study by MacMillan et al. (1996) in D79N mice, which carry a point mutation in the α_{2A}-AR gene, when the site of injection was the femoral instead of the carotid artery, the data could suggest that the α_{2A}-AR should be considered to be responsible for direct vasoconstriction. Furthermore, some of these differences could be species related, inasmuch as various studies have used mice, rats, or rabbits.

Why did norepinephrine produce a hypotensive response in the α_{2A}-AR knockouts (after α_1-AR blockade with prazosin) rather than no response at all? One explanation could be that, in the absence of any postsynaptic α-AR capable of eliciting vasoconstriction, the nonselective adrenergic agonist NE would stimulate only the vasodilatory β_2-adrenergic receptors on arterial smooth muscle. The same explanation could be given for the uniformly hypotensive responses elicited by NE in all six groups after combined blockade with prazosin and yohimbine. Yohimbine itself produced no change in BP baseline in the five groups, probably because its central presynaptic hypertensive effects in this case were balanced by the peripheral postsynaptic hypotensive ones. However, in the α_{2A}-AR knockouts, which lack the capacity for a central presynaptic hypertensive reaction to α_2-AR antagonists (Altman et al., 1999), a small hypotensive response due to blockade of either central α_{2B}-AR or any residual postsynaptic α_2-AR, although weak, became apparent.

In summary, these studies, by using sequential pharmacological manipulations of the α_2-AR subtypes in genetically altered mice deficient for each one of the α_2-AR subtypes, produced evidence suggesting that the α_{2A}-AR subtype is the one responsible for peripheral α_2-AR-mediated vasoconstictive responses. However, they also confirm that postsynaptic α-AR-mediated vasoconstriction is mainly (as much as 68%) a function of the α_1-AR.

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