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Effects of the selective cyclooxygenase-2 inhibitor nimesulide on vascular contractions in endothelium-denuded rat aorta

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Abstract

We have examined the effects of the selective cyclooxygenase-2 inhibitor nimesulide and the non-selective cyclooxygenase inhibitor indomethacin on vascular responsiveness of endothelium-denuded rat aorta. Isometric contractions were obtained to the α-adrenoceptor agonists phenylephrine (full agonist) and clonidine (partial agonist relative to phenylephrine) and to endothelin-1 and KCl. Maximum contractile responses to the partial agonist clonidine were significantly reduced by nimesulide (10 μM) and by indomethacin (10 μM) to 60.8 $\pm$ 8.5% ($n = 8$) and 69.0 $\pm$ 9.6% ($n = 12$) of control, respectively, as compared with the effects of vehicle (99.0 $\pm$ 5.8%; $n = 17$). The inhibitors had lesser effects against contractions to phenylephrine: nimesulide had no significant effect, whereas indomethacin caused a small but significant reduction in the maximum contraction to phenylephrine to 90.3 $\pm$ 5.0% ($n = 12$) of control (vehicle: 108.0 $\pm$ 5.2%, $n = 15$ nimesulide: 111.8 $\pm$ 5.9%, $n = 5$). Neither nimesulide nor indomethacin had any effect on contractions to endothelin-1 or KCl. These actions differed from the effects of the Ca^{2+} entry blocker nifedipine, which significantly reduced contractions to clonidine and KCl to a similar extent. The maximum contraction to clonidine was also significantly reduced by the thromboxane receptor antagonist SQ 29548 (1 μM) to 83.4 $\pm$ 6.4% of control ($n = 7$) (vehicle 115.5 $\pm$ 7.5%, $n = 7$). It is concluded that the cyclooxygenase inhibitors nimesulide or indomethacin reduce vascular responsiveness to α-adrenoceptor agonists in endothelium-denuded rat aorta, presumably by preventing the formation of vasoconstrictor prostaglandins in aortic smooth muscle by cyclooxygenase-2. This reduced vascular responsiveness was most clearly seen with the partial agonist clonidine.

Keywords: Nimesulide; Indomethacin; Cyclooxygenase; Aorta, rat; Nifedipine; Clonidine

1. Introduction

It is now well established that there are two forms of the enzyme cyclooxygenase: cyclooxygenase-1 and cyclooxygenase-2, key enzymes in the formation of prostanoids. Cyclooxygenase-1 is the constitutive form of the enzyme, present in gastric mucosa, the vascular endothelium and platelet; cyclooxygenase-2 is an inducible enzyme which can be induced in a wide variety of tissues (see the work of Hyslop and De Nucci (1993)). Nimesulide is a new selective inhibitor of cyclooxygenase-2, which shows low potency at inhibiting cyclooxygenase-1 (see the work of Tavares et al. (1995)). In contrast, indomethacin is relatively non-selective, inhibiting cyclooxygenase-1 and cyclooxygenase-2 over a similar concentration range; indomethacin and nimesulide have similar potencies at inhibiting cyclooxygenase-2 (Tavares et al., 1995).

Previous studies have reported that cyclooxygenase modulates vascular contractions in vitro, by endothelium-dependent and independent mechanisms. For instance, in aorta from spontaneously hypertensive rats (SHR), endothelium-dependent contractions to acetylcholine involve prostaglandin production by cyclooxygenase-1 (Ge et al., 1995), and an endothelium-independent thromboxane-like vasoconstrictor has also been reported (Dyer et al., 1994). Indomethacin improves endothelium-dependent relaxations in mesenteric arteries from SHR, presumably by blocking the production of vasoconstrictor prostaglandins (Luscher et al., 1990).

The object of this study was to investigate the possibility that vascular smooth muscle expresses cyclooxygenase-2, producing prostaglandins which are involved in modulating vasoconstrictor responses. We chose to investigate the cyclooxygenase-2 inhibitor nimesulide in compari-

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son with indomethacin to rule out involvement of cyclooxygenase-1. We examined the ability of nimesulide and indomethacin to affect vasoconstrictor responses in endothelium-denuded rat aorta, thus eliminating influences from the vascular endothelium. We chose to examine the effects of the two cyclooxygenase inhibitors against contractions to four vasoconstrictors: the α_1-adrenoceptor selective agonist phenylephrine, the α_1-adrenoceptor partial agonist clonidine, and endothelin-1 and KCl.

2. Methods

Male Wistar rats (200–250 g) were obtained from Trinity College Dublin.

2.1. Rat aorta

Aortic rings of 3–5 mm in length were gently rubbed to remove endothelium and attached to myograph transducers under 1 g tension in organ baths at 37°C in Krebs–Henseleit solution of the following composition (in mM): NaCl 119; $NaHCO_3$ 25; D-glucose 11.1; KCl 4.7; $CaCl_2$ 2.5; KH_2PO_4 1.2; $MgSO_4$ 1.0; EDTA 0.03; ascorbic acid 0.28. Additionally, cocaine (3 μM), corticosterone (30 μM), and propranolol (3 μM) were present.

Tissues were contracted with phenylephrine (1 μM), exposed to acetylcholine (10 μM) to test for endothelium-dependent relaxations, and washed. Tissues which relaxed to acetylcholine were discarded due to the presence of a functional endothelium. Bathing fluid was then changed every 15 min for the next hour. Tissues were then contracted with phenylephrine administered cumulatively in 0.5 log unit increments beginning with 1 nM. Once a maximum response to phenylephrine had been obtained, tissues were washed and bathing fluid was changed every 15 min for another 60 min. Following 60-min exposure to nimesulide (1, 10 or 100 μM), indomethacin (1 or 10 μM) or vehicle, tissues were again contracted with phenylephrine administered cumulatively in 0.5 log unit increments beginning with 1 nM. Separate experiments examining contractions to clonidine and KCl were carried out as described for phenylephrine. In some experiments, the effects of 1-h exposure to nifedipine (0.1 μM) or the thromboxane A_2 receptor antagonist SQ 29548 (1 μM) were examined against contractions to clonidine. Experiments employing endothelin-1 differed in that it proved difficult to wash out the agonist. Hence, in these experiments, a control concentration–response curve was obtained to noradrenaline, followed by washout, and following 1-h exposure to test drug or vehicle, a concentration–response curve was obtained to endothelin-1. The maximum response to endothelin-1 was expressed as a percentage of the maximum response obtained to noradrenaline in the control concentration–response curve.

To investigate the subtype of α-adrenoceptor activated in rat aorta, experiments were carried out in which the second concentration–response curve to clonidine was obtained following 1-h exposure to the α_1-adrenoceptor selective antagonist prazosin (0.01 μM), the α_2-adrenoceptor selective antagonist yohimbine (10 μM), or vehicle.

2.2. Drugs

Acetylcholine chloride (Sigma, Poole, UK); clonidine hydrochoride (Research Biochemicals, Natick, USA); cocaine hydrochloride (Sigma); corticosterone (RBI); endothelin 1 (Sigma); indomethacin (RBI); nifedipine (Sigma) nimesulide (gift: Helsinn-Birex, Ireland); phenylephrine hydrochloride (RBI); prazosin hydrochloride (Sigma); propranolol hydrochloride (Sigma); prostaglandin $F_{2\alpha}$ (Sigma); SQ 29548 ([1*S*-[1α,2α(*Z*),3α,4α]]-7-[3-[[2-[(phenylamino)carbonyl]hydrazino]methyl]-7-oxabicyclo[2.2.1]hept-2-yl]-5-heptanoic acid; RBI); yohimbine hydrochloride (Sigma).

Drugs were dissolved in distilled water, except for corticosterone, indomethacin, nifedipine and nimesulide (100% ethanol), and dilutions were made up in distilled water.

2.3. Statistics

Values are arithmetic mean ± S.E.M. pD_2 ($-\log EC_{50}$) values were obtained using the GraphPad Prism program for PC. Antagonist potency was expressed as a pK_B from the equation $pK_B = B + \log(DR - 1)$, where B is the antagonist concentration ($-\log M$), and DR is the agonist dose ratio. pD_2 and maximum contractile responses were compared between groups using Student's *t*-test, or analysis of variance and Dunnett's test (Instat Program), where appropriate.

3. Results

3.1. Contractions to phenylephrine

Phenylephrine produced isometric contractions with a pD_2 value of 7.59 ± 0.05 ($-\log M$) and a maximum contraction of 0.84 ± 0.04 g ($n = 33$ vessels from 18 rats), in endothelium-denuded rat aorta (results from the first, control, concentration–response curve). Phenylephrine produced isometric contractions with pD_2 values of 7.30 ± 0.08, 7.05 ± 0.10 and 7.31 ± 0.08 ($-\log M$) and a maximum contraction of 108.0 ± 5.2% ($n = 15$), 111.8 ± 5.9% ($n = 5$) and 90.3 ± 5.0% of control ($n = 12$) following 1-h exposure to vehicle, nimesulide (10 μM) or indomethacin (10 μM), respectively (see Fig. 1). Neither nimesulide nor indomethacin significantly reduced potency of phenylephrine, but indomethacin slightly but significantly reduced the maximum response to phenylephrine ($P <$

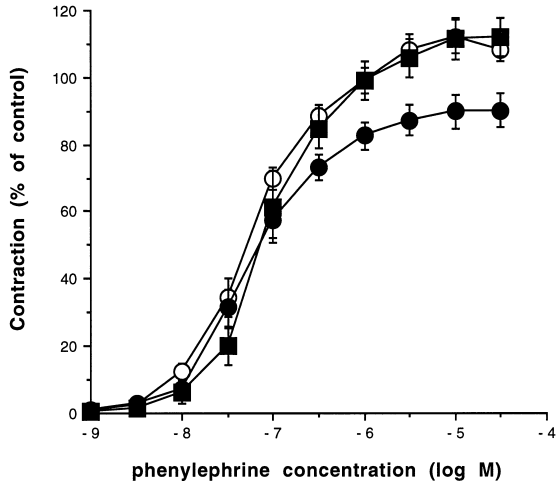

Fig. 1. Concentration–contractile response curves obtained to phenylephrine in aortic rings from rats following 1 h exposure to nimesulide (10 μM) (■), indomethacin (10 μM) (●) or vehicle (○). Values are isometric contraction (% of control) and are the mean of at least five experiments. Vertical bars represent S.E.M.

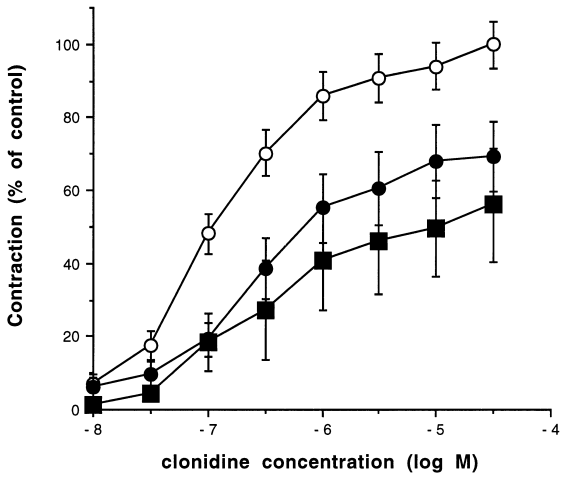

Fig. 2. Concentration–contractile response curves obtained to clonidine in aortic rings from rats following 1 h exposure to nimesulide (10 μM) (■), indomethacin (10 μM) (●) or vehicle (○). Values are isometric contraction (% of control) and are the mean of at least five experiments. Vertical bars represent S.E.M.

0.05: analysis of variance and Dunnett's multiple comparison test).

3.2. Contractions to clonidine

Clonidine produced isometric contractions with a pD_2 value of 6.96 ± 0.04 ($-\log M$) and a maximum contraction of 0.57 ± 0.03 g ($n = 44$ vessels from 22 rats), in endothelium-denuded rat aorta (results from the first, control, concentration–response curve). The α-adrenoceptor antagonists prazosin (0.01 μM) and yohimbine (10 μM) significantly shifted the potency of clonidine, without affecting the maximum response. The prazosin and yohimbine pK_B values were 9.34 ± 0.19 ($n = 4$) and 6.46 ± 0.11 ($n = 6$), respectively, indicative of α_1-adrenoceptors, suggesting that clonidine acts predominantly on α_1-adrenoceptors. Clonidine produced isometric contractions with pD_2 values of 6.84 ± 0.10, 6.40 ± 0.32 and 6.52 ± 0.15 ($-\log M$) and a maximum contraction of $99.0 \pm 5.8\%$ ($n = 17$), $60.8 \pm 8.5\%$ ($n = 8$) and $69.0 \pm 9.6\%$ of control ($n = 12$) following 1-h exposure to vehicle, nimesulide (10 μM) or indomethacin (10 μM), respectively (see Fig. 2). Nimesulide (10 μM) or indomethacin (10 μM) significantly reduced the maximum response to clonidine as compared to the maximum response obtained in vehicle experiments ($P < 0.01$ and $P < 0.05$, respectively: analysis of variance and Dunnett's multiple comparison test), without significantly affecting potency. Indomethacin (1 μM) and nimesulide (1 μM) did not significantly affect the maximum response to clonidine ($110.0 \pm 7.4\%$, $n = 4$ and $115.7 \pm 22.8\%$ of control, $n = 4$, respectively). Nimesulide (100 μM) reduced the maximum contraction to clonidine to $18.4 \pm 18.4\%$ of control ($n = 5$), which was significantly less than the $123.2 \pm 24.1\%$ of control ($n = 7$) obtained after vehicle (ethanol 100%).

3.3. Contractions to KCl

KCl produced isometric contractions with an EC_{50} value of 17.9 ± 1.4 mM and a maximum contraction of 0.61 ± 0.04 g ($n = 25$ vessels from 16 rats), in endothelium-denuded rat aorta (results from the first, control, concentration–response curve). KCl produced isometric contractions with EC_{50} values of 15.9 ± 1.9, 22.2 ± 2.0 and 18.1 ± 1.8 mM and a maximum contraction of $115.3 \pm 4.6\%$ ($n = 10$), $109.7 \pm 3.0\%$ ($n = 6$) and $112.4 \pm 6.2\%$ of control ($n = 7$) following 1-h exposure to vehicle, nimesulide (10 μM) or indomethacin (10 μM), respectively (see Fig. 3). Nimesulide and indomethacin failed to affect significantly either the maximum response to, or the potency of, KCl.

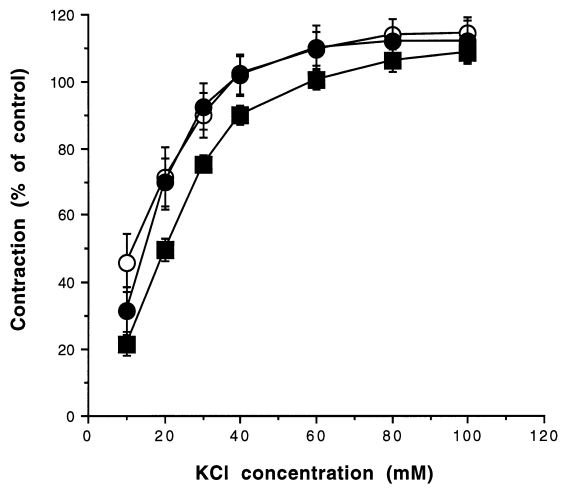

Fig. 3. Concentration–contractile response curves obtained to KCl in aortic rings from rats following 1 h exposure to nimesulide (10 μM) (■), indomethacin (10 μM) (●) or vehicle (○). Values are isometric contraction (% of control) and are the mean of at least five experiments. Vertical bars represent S.E.M.

3.4. Contractions to endothelin-1

Endothelin-1 produced isometric contractions with a pEC_{50} value of 8.49 ± 0.12 and a maximum contraction of $101.7 \pm 13.8\%$ of the maximum obtained to noradrenaline in the control concentration–response curve ($n = 5$), in endothelium-denuded rat aorta (results from the second, vehicle, concentration–response curve). Endothelin-1 produced isometric contractions with pEC_{50} values of 8.63 ± 0.05 and 8.71 ± 0.10 and a maximum contraction of $98.8 \pm 1.0\%$ ($n = 4$) and $103.6 \pm 17.8\%$ ($n = 5$) of the maximum obtained to noradrenaline in the control concentration–response curve, following 1-h exposure to nimesulide (10 μM) or indomethacin (10 μM), respectively. Nimesulide and indomethacin failed to affect significantly either the maximum response to, or the potency of, endothelin-1.

3.5. Effects of nifedipine

The Ca^{2+} entry blocker nifedipine (0.1 μM) significantly reduced contractions to KCl to $42.3 \pm 8.5\%$ of control ($n = 4$), as compared to the effects of vehicle ($97.8 \pm 8.0\%$, $P < 0.05$). Nifedipine (0.1 μM) significantly reduced contractions to clonidine to $49.9 \pm 12.9\%$ of control ($n = 4$), as compared to the effects of vehicle ($105.4 \pm 8.2\%$, $P < 0.05$). Nifedipine reduced maximum contractions to KCl and clonidine to a similar extent. In contrast, both nimesulide (10 μM) and indomethacin (10 μM) significantly reduced contractions to clonidine without significantly affecting contractions to KCl (compare Figs. 2 and 3).

3.6. Effects of SQ 29548

The thromboxane receptor antagonist SQ 29548 (1 μM) did not significantly affect the potency of clonidine but significantly reduced the maximum response to $83.4 \pm 6.4\%$ of control ($n = 7$), as compared with the effects of vehicle ($115.5 \pm 7.5\%$ of control, $n = 7$; $P < 0.01$). The same concentration of SQ 29548 significantly reduced the maximum response to prostaglandin $F_{2\alpha}$ to $12.4 \pm 3.7\%$ of control ($n = 4$), as compared with the effects of vehicle ($114.1 \pm 8.3\%$ of control, $n = 4$; $P < 0.05$).

4. Discussion

In this study, we have examined the effects of the selective cyclooxygenase-2 inhibitor nimesulide on vascular function in rat aorta, in comparison with the effects of the non-selective cyclooxygenase inhibitor indomethacin. To eliminate any complications due to cyclooxygenase in the vascular endothelium which may produce vasodilator and vasoconstrictor prostaglandins, vessels were endothelium-denuded.

Nimesulide and indomethacin (both 10 μM) were approximately equi-effective at reducing contractions to clonidine in rat aorta (present results). These concentrations were the first effective concentrations of both agents, since the lower concentration of 1 μM did not produce any effect. However, indomethacin is 100–1000 times more potent than nimesulide against cyclooxygenase-1, but approximately equi-potent at cyclooxygenase-2 (Berti et al., 1990; Tavares et al., 1995). Nimesulide is reported to be ineffective in vitro against cyclooxygenase-1 at concentrations of 100 μM, whereas indomethacin produces inhibition at concentrations below 1 μM (Tavares et al., 1995). Against cyclooxygenase-2 assays, nimesulide was effective at concentrations of 10 μM, and was only slightly less potent than indomethacin (Tavares et al., 1995). Given the similar potencies of nimesulide and indomethacin in rat aorta, it seems likely, therefore, that these agents act to prevent the formation of vasoconstrictor prostaglandins by cyclooxygenase-2 in vascular smooth muscle. However, it is not clear whether this cyclooxygenase-2 was constitutive or induced in aortic smooth muscle. Since control contractions to clonidine were obtained approximately 1.5 h after beginning experiments, and since these were inhibited by nimesulide approximately 3 h after beginning the experiment, it is unclear whether the cyclooxygenase-2 enzyme can be induced in such a short time in response to the surgical trauma of removing the aorta, or whether it is constitutively expressed: it has been shown that cyclooxygenase-2 is constitutively expressed in rat liver (Belton et al., 1997). In rat aorta, induction of nitric oxide synthase can be shown to occur over a longer time period (6 h; Moritoki et al., 1992). However, in cultured rat aortic smooth muscle cells, stimulation with serum resulted in cyclooxygenase-2 expression within 45 min, reaching a peak at 90 min (Rimarachin et al., 1994).

The possibility remains that indomethacin and nimesulide act by another as yet unidentified mechanism to reduce contractions to clonidine. Since contractions to KCl were unaffected by nimesulide (10 μM), it is unlikely that the actions of nimesulide against clonidine involve Ca^{2+} entry blockade, although it has previously been suggested that indomethacin may have Ca^{2+} antagonistic actions (Northover, 1977). The Ca^{2+} entry blocker nifedipine affected equally contractions to KCl and clonidine, whereas both nimesulide and indomethacin significantly reduced contractions to clonidine without affecting those to KCl. It is more likely that direct receptor mediated actions of α-adrenoceptor agonists, but not membrane depolarisation by KCl, stimulate production of predominantly vasoconstrictor prostaglandins. The failure of nimesulide to affect contractions to phenylephrine, and the relatively small effect of indomethacin against phenylephrine, contrast with the marked effects against clonidine. These differences may be due to the partial agonistic properties of clonidine at α_1-adrenoceptors in this preparation, in contrast to those of the full agonist phenylephrine: submaximal responses to

a weak partial agonist may be more easily reduced than those to a full agonist. Contractions to clonidine are potently antagonised by prazosin in rat aorta (O'Rourke et al., 1995), suggesting that clonidine acts mainly via α_1-adrenoceptors. In the present study, the potency of the α_1-adrenoceptor selective antagonist prazosin (pK_B of 9.34) and of the α_2-adrenoceptor selective antagonist yohimbine (pK_B of 6.46) suggest that contractions to clonidine in rat aorta involve mainly α_1-adrenoceptors (see the work of Docherty (1989)). However, we cannot rule out the possibility that a component of the response to clonidine is α_2-adrenoceptor mediated. An α_2-adrenoceptor is expressed in rat aorta (Ping and Faber, 1993) and may be a target for the test agent chloroethylclonidine (O'Rourke et al., 1997). Whichever is the case, it is clear that clonidine is a useful agent to assess such effects of cyclooxygenase inhibitors. We also assessed the effects of the thromboxane A_2 receptor antagonist SQ 29548 against contractions to clonidine. SQ 29548 (1 μM) did not affect the potency but significantly reduced the maximum response to clonidine. Concentrations of SQ 29548 of 0.01 μM shift the potency of 8-epi prostaglandin $F_{2\alpha}$ in porcine coronary artery (Kromer and Tippins, 1996), and a SQ 29548 of 1 μM virtually abolished contractions to prostaglandin $F_{2\alpha}$ in the present study. These results with SQ 29548 suggest that the predominantly vasoconstrictor prostaglandins produced in response to clonidine may act on the thromboxane TP receptor to produce contractions.

One difference between indomethacin and nimesulide found in this study was that indomethacin produced a small but significant reduction in the response to phenylephrine. This may suggest that indomethacin has effects in addition to those of nimesulide, but in a previous study from this laboratory indomethacin (10 μM) did not affect the maximum contraction to noradrenaline (Belton et al., 1997).

Endothelin-1 has been reported to stimulate formation of thromboxane A_2 in rat aorta, so that contractions are reduced by indomethacin (Reynolds and Mok, 1990). However, in our studies of endothelium-denuded aorta, there was no effect of indomethacin or nimesulide on contractions to endothelin. Indomethacin (28 μM) has been reported to decrease potency of 5-HT in rat aorta (Porsa et al., 1989), and indomethacin (5 μM) has been reported to reduce the maximum response to KCl in the perfused mesenteric arterial bed from male but not female rats, and to enhance contractions to KCl in the venous bed from female rats (Le Marquer-Domagala and Finet, 1997). These studies are not directly comparable to the present study, particularly as the endothelium was presumably intact, although the concentrations of indomethacin used are similar. Other authors have reported that endothelin-1 increases the release of prostaglandin E_2 from endothelium-denuded porcine coronary artery: indomethacin increased the contractile response to endothelin-1 by preventing the inhibitory actions of prostaglandin E_2 (Suzuki et al., 1992). The presence of a non-endothelial thromboxane-like constrictor agent has been reported in the aorta of the SHR (Dyer et al., 1994) and of rats made hypertensive by aortic coarctation (Lin et al., 1994). In one study, cyclooxygenase-2 has been implicated in modulation of the contractile response to histamine in rat trachea, although in that study an increased maximum contractile response was reported and in response to relatively low concentrations of cyclooxygenase-2 inhibitor (Charette et al., 1995).

In conclusion, the cyclooxygenase inhibitors nimesulide or indomethacin reduce vascular responsiveness to α-adrenoceptor agonists in endothelium-denuded rat aorta, presumably by preventing the formation of predominantly vasoconstrictor prostaglandins in aortic smooth muscle by cyclooxygenase-2. This reduced vascular responsiveness was most clearly seen with the partial agonist clonidine.

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