SELECTIVE ANTAGONISM OF THE HYPOTENSIVE EFFECTS OF DOPAMINE AGONISTS IN SPONTANEOUSLY HYPERTENSIVE RATS

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SUMMARY Agonists of dopamine receptors can lower blood pressure by vasodilation through action on dopamine receptors, inhibition of sympathetic nerve activity by action on dopamine receptors, or actions in the central nervous system. Fenoldopam, a selective dopamine agonist, piribedil, a selective dopamine agonist, and dipropyl dopamine, a mixed dopamine and dopamine agonist, were injected intravenously in pentobarbital-anesthetized, spontaneously hypertensive rats (SHR). The mechanism for the antihypertensive effect was evaluated by administration of the selective dopamine agonist SCH 23390 and the selective dopamine agonist domperidone. While SCH 23390 only antagonized the hypotensive effects of fenoldopam, domperidone abolished the fall in blood pressure produced by dipropyl dopamine and piribedil but not by fenoldopam. Increments in heart rate and plasma norepinephrine levels accompanied the hypotensive effects of fenoldopam. The increase in heart rate was abolished by a dose of SCH 23390 sufficient to completely block the hypotensive effects and was significantly attenuated by the ganglionic blocking agent hexamethonium, which suggests that the increase in heart rate was due to a baroreceptor reflex. Fenoldopam does not cross the blood-brain barrier, which suggests that its hypotensive effect was mediated by peripheral dopamine receptors. Since domperidone does not cross the blood-brain barrier and significantly antagonized the hypotensive and bradycardic effects of dipropyl dopamine and piribedil, these effects were mediated primarily by peripheral dopamine receptors. These results indicate that SCH 23390 and domperidone are useful agents to identify the receptor subtype mediating the action of dopamine agonists in SHR. (Hypertension 8: 298–302, 1986)

KEY WORDS • dopamine receptors • dopamine antagonists • SCH 23390 • domperidone • fenoldopam • piribedil • dipropyl dopamine

DOPAMINE (DA) receptors have been divided into two subtypes, DA, and DA,. The DA, receptors are located in the vascular smooth muscle and subserve vasodilation, primarily in the renal and other visceral vascular beds. The DA, receptors are located on postganglionic sympathetic neurons and, when activated, inhibit norepinephrine (NE) release from sympathetic nerve terminals, leading indirectly to vasodilation and a fall in heart rate (HR).Because of these actions, agonists of both receptor subtypes are receiving increasing attention as potential antihypertensive agents. Dopamine and most DA agonists act on both DA receptor subtypes and on other receptors in the central nervous system and periphery. Therefore, it is important to develop an animal model to separate these diverse mechanisms. Several studies have demonstrated that agonists, which may act on DA, or DA, receptors, lower blood pressure (BP) in spontaneously hypertensive rats (SHR). Until recently, selective antagonists were not available to prove that reduction in BP was due to action on either one or both receptor subtypes. The benzazepine SCH 23390 and the butyrophenone domperidone are extremely selective antagonists of DA, and DA, receptors, respectively. The present experiments were designed to determine whether these selective antagonists inhibit the hypotensive ef-
fected of three DA agonists: fenoldopam, a selective DA_1 agonist, piribedil, a relatively selective DA_2 agonist, and dipropyl DA (DPDA), which acts on both DA_1 and DA_2 receptors.

Materials and Methods

Male SHR were obtained from Charles River Laboratory (Wilmington, MA, USA) and weighed between 290 and 350 g. The animals were anesthetized with pentobarbital, 60 mg/kg intraperitoneally, and 3 mg of pentobarbital was given intraperitoneally as needed. The animals were killed at the end of the experiment. A tracheotomy was performed on all animals. The left carotid artery was catheterized with polyethylene-50 tubing for monitoring mean arterial pressure (MAP) and blood sampling. Both external jugular veins were catheterized with polyethylene-50 tubing for drug administration. The MAP was measured with a Bell and Howell Model 4-327-1 transducer (Pasadena, CA, USA), HR was obtained with a Beckman Model 9857B cardiotachometer (Schiller Park, IL, USA), and both were recorded on a Beckman Model R dynograph.

After MAP and HR had stabilized, fenoldopam, 40 μg/kg, DPDA, 5 μg/kg, and piribedil, 20 μg/kg, were administered by rapid intravenous injections in six SHR. The order of administration was varied, and MAP and HR were allowed to return to baseline between injections. An infusion of SCH 23390 was begun at 0.15 μg/min. Ten minutes later while SCH 23390 was still being infused, injections of fenoldopam, DPDA, and piribedil were repeated.

In five SHR, the same protocol was followed except that domperidone was administered in a dose of 100 μg/kg. The agonist injections were repeated 10 minutes after the injection of domperidone.

The effect of fenoldopam on arterial plasma NE levels was determined in five SHR. After stabilization of HR and MAP for 10 minutes, a 1-ml sample of arterial blood was drawn for determination of baseline NE levels. Ten minutes later, MAP and HR had equilibrated and fenoldopam, 40 μg/kg, was given by intravenous injection. An arterial sample was drawn 1 minute after the injection, which coincided with the maximum HR. SCH 23390 was infused at 0.15 μg/min, and NE levels were obtained after 10 minutes of constant infusion. A final sample for NE determination was obtained 1 minute after a 40 μg/kg injection of fenoldopam during the SCH 23390 infusion. Arterial blood was collected in heparinized tubes and stored at 4°C until separation. Plasma was then stored at -80°C until NE levels were determined. The NE was measured by reverse phase high-performance liquid chromatography using electrochemical detection, as previously described.

The effects of fenoldopam on MAP and HR were recorded before and after infusion of the ganglionic blocking agent hexamethonium in five SHR. After the responses to fenoldopam had been recorded, hexamethonium was infused during a 30-minute period in a dose of 15 to 20 mg/kg, which blocked the tachycardia induced by the ganglionic stimulating agent dimethyl-4-phenylpiperazinium, 20 μg/kg i.v. During administration of hexamethonium, saline solution was infused to maintain MAP. A constant infusion of hexamethonium, 0.15 mg/min, was continued. Injections of dimethyl-4-phenylpiperazinium and fenoldopam were repeated. In five SHR, fenoldopam, 40 μg/kg, and diazoxide, 3 mg/kg, were given by intravenous injection before and during the infusion of SCH 23390, 15 μg/min.

Data are presented as means ± SEM. The paired t test was used for analysis. Results were considered significant if the p value was less than 0.05.

Results

The effects of fenoldopam, DPDA, and piribedil before and after administration of SCH 23390 are shown in Figure 1. Before the infusion of SCH 23390, 0.15 μg/min, MAP was 163 ± 3 mm Hg. Fenoldopam decreased MAP by 55 ± 3 mm Hg, an effect that lasted for 8 to 12 minutes. Piribedil and DPDA decreased MAP by 46 ± 3 and 36 ± 4 mm Hg, respectively. The hypotensive effect of DPDA was transient and lasted from 1 to 2 minutes, while that of piribedil lasted from 8 to 12 minutes. Figure 2 illustrates the effects of fenoldopam, DPDA, and piribedil on MAP and HR in an anesthetized spontaneously hypertensive rat.

Although MAP was not significantly changed by infusion of SCH 23390, the hypotensive response of fenoldopam was significantly attenuated during the
SCH 23390 infusion. The MAP decreased by only 7 ± 1 mm Hg. In contrast, the increase in HR produced by fenoldopam was reduced only from 24 ± 3 to 19 ± 3 beats/min. As shown in Figure 1, SCH 23390 infusion did not affect the hypotensive effects of or the HR responses to DPDA and piribedil. Both DPDA and piribedil decreased HR. The DPDA decreased HR by an average 11 ± 2 beats/min, while piribedil decreased HR by 31 ± 4 beats/min.

Before domperidone administration, average MAP was 171 ± 3 mm Hg. Domperidone, 100 μg/kg i.v., did not affect MAP. Fenoldopam decreased MAP by an average of 30 ± 2 mm Hg and increased HR by 19 ± 3 beats/min. Neither response was affected by domperidone administration (Figure 3). Piribedil decreased MAP by 29 ± 4 mm Hg, and DPDA decreased MAP by 27 ± 3 mm Hg; however, these effects were abolished by administration of domperidone (see Figure 3).

As in the previous series of experiments with SCH 23390, piribedil decreased HR more than did DPDA despite equal hypotensive effects. Piribedil reduced average HR by 28 ± 4 beats/min, and DPDA reduced average HR by 11 ± 1 beats/min. Domperidone administration completely eliminated the reduction in HR by DPDA. In contrast, after domperidone administration, piribedil decreased HR by 14 ± 4 beats/min. Accordingly, despite the complete elimination of the hypotensive effects of piribedil, the bradycardic effect remained. The effects of the agonists on BP were less in this series of experiments than in the previous series; however, the effects on HR were similar.

Because in the initial studies SCH 23390 did not completely eliminate the increase in HR produced by fenoldopam, additional experiments were conducted to elucidate possible mechanisms. Experiments were performed in five SHR to obtain arterial NE levels. Levels of NE were not changed significantly by infusion of SCH 23390, 0.15 μg/min. Administration of fenoldopam, 40 μg/kg, increased plasma NE levels in each rat. The average plasma NE levels increased significantly from 0.185 ± 0.036 to 0.375 ± 0.065 ng/ml. During SCH 23390 infusion, fenoldopam administration again increased plasma NE levels in each animal. The average plasma NE level after SCH 23390 infusion was 0.255 ± 0.05 ng/ml, and this value increased to 0.508 ± 0.047 ng/ml after administration of fenoldopam.

These data suggested that the hypotensive effects remaining after SCH 23390 infusion were sufficient to cause baroreceptor-mediated increments in HR. Accordingly, two additional studies were conducted. To investigate the baroreceptor mechanism further, fenoldopam was administered before and during constant infusion of the ganglionic blocking agent hexamethonium in five SHR. The average MAP was 164 ± 8 mm Hg before hexamethonium infusion and 130 ± 7 mm Hg after hexamethonium. The average HR before hexamethonium infusion was 383 ± 10 beats/min, and was reduced to 298 ± 9 beats/min after hexamethonium. The tachycardic response to the ganglionic stimulant dimethyl-4-phenylpiperazinium was almost completely eliminated by hexamethonium infusion. Fenoldopam reduced MAP by an average of 26 ± 2% before hexamethonium infusion and 20 ± 3% after hexamethonium. Before hexamethonium infusion, fenoldopam administration increased HR by 25 beats/min, respectively (*p < 0.05, **p < 0.001).
± 2 beats/min; after hexamethonium infusion, the rise in HR was markedly reduced to 8 ± 1 beats/min, which was not significantly different from the HR recorded before fenoldopam.

Finally, fenoldopam was administered before and during infusion of SCH 23390, 15 µg/min. This infusion rate virtually abolished both the increase in HR and the decrease in MAP produced by fenoldopam without affecting the reduction in BP and the increase in HR produced by the vasodilating agent diazoxide, which was used as a positive control (Figure 4).

**Discussion**

The present study confirmed previous reports that DA₁ and DA₂ antagonists are effective in decreasing BP in the anesthetized SHR and demonstrated that these hypotensive effects can be selectively antagonized by the DA₁ antagonist SCH 23390 and the DA₂ antagonist domperidone. These antagonists make possible the use of anesthetized SHR to determine the hypotensive mechanisms of putative DA agonists. These studies were conducted in anesthetized SHR because centrally acting DA agonists, such as piribedil, can produce pronounced behavioral effects in unanesthetized rats, including stereotypy and hyperactivity, which could confound the results. Previous studies with fenoldopam have indicated that this compound is a selective DA₁ agonist that decreases BP by vasodilating renal, mesenteric, and possibly other vascular beds. Hahn et al. reported that fenoldopam does not cross the blood-brain barrier; thus, unless the compound acts at areas not protected by this barrier, a central mechanism can be ruled out. In the present study, fenoldopam consistently decreased BP and this response was antagonized by SCH 23390, which confirms the DA₁ receptor-mediated mechanism. As expected with vasodilating agents, the hypotension produced by fenoldopam was accompanied by increments in HR and plasma NE levels. The increase in HR produced by fenoldopam was not blocked by an infusion of SCH 23390, 0.15 µg/min, but was eliminated when a sufficiently high dose of SCH 23390 was administered to abolish the hypotensive effect. The baroreceptor-mediated increase in HR also was suggested by the hexamethonium-induced antagonism of this effect. Increments in HR previously have been observed with orally administered fenoldopam in hypertensive patients. Furthermore, in a recent study, we observed that intravenous infusions of fenoldopam in hypertensive patients consistently increased plasma NE levels.

Although piribedil is used primarily as a centrally acting DA agonist for the treatment of Parkinson's disease, it also inhibits adrenergic transmission by action on DA₂ receptors. In the present study, the hypotensive effects of piribedil were completely blocked by domperidone, whereas the bradycardic effect was significantly but not completely antagonized. Since domperidone does not cross the blood-brain barrier, these data suggest that a component of the bradycardic response could have been due to a central action, although a peripheral effect, possibly by a vagal mechanism, was not ruled out.

In contrast, both the hypotension and bradycardia produced by DPDA were completely antagonized by domperidone, which suggests that the effects of DPDA were entirely peripheral in these experiments. These data also indicate that the primary mechanism for the hypotensive effect of DPDA is action on peripheral DA₂ receptors and that the DA₁ component of this drug was not involved in the dose used. This conclusion is supported by the lack of effect of SCH 23390 on DPDA-induced hypotension. Similar results were obtained by Cavero et al., who found that the bradycardia and hypotension produced by DPDA in the normotensive rat also were blocked by domperidone. In contrast to these responses in the rat, Taylor et al. considered that propylbutyl-DA, which resembles DPDA pharmacologically in the dog, decreases BP by acting on both DA₁ and DA₂ receptors in the hypertensive patient. This conclusion was based on the demonstration that low doses of metoclopramide decreased the hypotensive effect of propylbutyl-DA but did not affect the renal vasodilatation produced by the drug. When larger doses of metoclopramide were administered, a greater block of the hypotensive effect occurred and the renal vasodilating response was antagonized.

Although our study primarily implicates a peripheral mechanism for the hypotensive effects of DPDA and piribedil, a central mechanism may be the site of action...
for other DA agonists. Nagahama et al. reported that the hypotensive effects of bromocriptine in conscious SHR were not antagonized by domperidone but were blocked by metoclopramide, which crosses the blood-brain barrier. In contrast, the hypotensive effect of bromocriptine in the anesthetized dog has been attributed to a peripheral mechanism, which suggests a possible species difference between the SHR and the dog. Since both bromocriptine and metoclopramide are not selective and act on DA and other receptors, further studies are required to prove that the hypotensive effects of bromocriptine are solely the result of action on central DA receptors.

Finally, the excellent correlation between the hypotensive effects of DA, and D₂ agonists in the anesthetized SHR and in hypertensive patients suggests that the SHR are suitable for screening new DA, and D₂ agonists for potential clinical use as antihypertensive agents.

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