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In vivo effects of fenoldopam on autonomic nervous system after inhibition or activation of ganglionic transmission

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Abstract

The study investigated the effects of dopamine D1-like receptor stimulation on the autonomic nervous system. Fenoldopam (20 μg/kg) was injected i.v. in conscious sinoaortic denervated dogs, that is, surgically deprived of baroreflex pathways. In barodenervated dogs, fenoldopam (20 μg/kg) induced arterial hypotension as well as bradycardia and reduced noradrenaline plasma levels. Pentolinium (0.1 mg/kg i.v.), used to induce partial blockade of nicotinic ganglionic receptors, suppressed the fenoldopam-induced decrease in sympathetic tone, suggesting a ganglionic location for the dopamine D1-like receptor. Moreover, the inability of fenoldopam to reduce the nicotine-induced increase in sympathetic tone suggests that a postsynaptic ganglionic location can be excluded for the dopamine D1-like receptor. The results of these ''in vivo'' experiments strongly suggest a presynaptic location for the ganglionic dopamine D1-like receptor, stimulation of which results in a reduction of sympathetic tone.

Keywords: Dopamine D1-like receptor; Ganglion; Fenoldopam; Sympathetic tone

1. Introduction

Several studies have shown that dopamine plays a major role in the cardiovascular system. Dopamine receptors are classified into two families (Jose et al., 2002). One family consists of the ''dopamine D1-like'' receptors, two of which have been cloned from mammals (D1 and D5). The other family consists of the ''D2-like'' receptors, three of which have been cloned: D2, D3 and D4 receptors. In the cardiovascular system, vascular dopamine D1-like receptor stimulation by a selective agonist such as fenoldopam induces vasodilatation. In normal animals, this vasodilating effect is associated with reflex tachycardia and an increase in sympathetic tone. Concerning the presence of dopamine D1-like receptors in the sympathetic nervous system, conflicting results have been reported about their existence at the ganglionic level. Several authors (Kimura et al., 1992; Kohli et al., 1988; Satoh et al., 1989) have suggested that dopamine D1-like receptors are absent in the dog stellate ganglion. In contrast, others (Sabouni et al., 1986; Shebuski et al., 1985) have shown that dopamine D1-like receptor agonists inhibit ganglionic transmission in the dog cardiac sympathetic ganglia.

Previous work from our group has shown that fenoldopam induces both bradycardia and a decrease in noradrenaline plasma levels in animals deprived of baroreflex pathways, that is, sinoaortic denervated dogs (Damase-Michel et al., 1995). The aim of the present study was to analyse the mechanism of the decrease in sympathetic tone elicited by fenoldopam, a specific dopamine D1-like receptor agonist (Nichols et al., 1990) in sinoaortic denervated dogs. This model has been used for the study of hypertensive drugs (Damase-Michel et al., 1987, 1989) and of another dopamine-receptor agonist, quinpirole (Damase-Michel et al., 1990).

2. Materials and methods

2.1. General protocol

Experiments were performed on beagle sinoaortic denervated dogs (male, 11–16 kg). The dogs were trained to stay

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still for 2 h on a Pavlov table and accustomed to i.v. infusion and blood sampling.

Several months before the different experimental protocols, sinoaortic denervation was performed. Briefly, as already described, carotid and aortic nerves were cut under chloralose anaesthesia (80 mg/kg i.v.) during two successive procedures, one at time 0 and the second one 7 weeks later (Damase-Michel et al., 1987). During surgery, care was taken to keep intact both vagal and sympathetic fibres of the vagus. The effectiveness of baroreceptor denervation was checked by the failure of noradrenaline (2 μg/kg i.v.) or phenylephrine (0.1, 1.0 and 10.0 μg/kg i.v.) to induce bradycardia and of nitroglycerin (1, 3, 10 and 30 μg/kg i.v.) to induce tachycardia.

Systolic and diastolic blood pressure and heart rate were recorded by means of a catheter introduced into the abdominal aorta via the femoral artery. Noradrenaline plasma levels were measured after blood collection from the femoral artery.

Drugs were injected peripherally through a catheter introduced into the radial vein.

The European Community guidelines for the use of experimental animals were adhered to throughout the experiments.

2.2. Experimental protocols

2.2.1. Effect of fenoldopam (20 μg/kg i.v.) in sinoaortic denervated conscious dogs

In a first step, we checked that fenoldopam (20 μg/kg i.v.) produced a response similar to that obtained in previous experiments (Damase-Michel et al., 1995). A pool of four conscious, sinoaortic denervated, male beagle dogs was used for these experiments and those on ganglionic blockade or stimulation. These animals had been denervated for less than 4 months. They were characterised by a systolic blood pressure around 210 mm Hg and a diastolic blood pressure close to 120 mm Hg. Noradrenaline plasma levels were around 500 pg/ml.

Systolic blood pressure, diastolic blood pressure and heart rate as well as noradrenaline plasma levels were measured 5 min before fenoldopam or NaCl (0.9%) injection ($t0$) and at $t+2$, $t+5$ and $t+15$ after fenoldopam ($n=8$) or NaCl 0.9% ($n=6$) injection. A delay of at least 1 week was allowed between two experiments on the same dog.

2.2.2. Ganglionic blockade

Ganglionic blockade was obtained using pentolinium, a peripheral ganglionic blocker that does not cross the blood-brain barrier. According to the data of Gaspo et al. (1994), we determined the dose of pentolinium (ganglioplegic drug) which induces a partial ganglionic blockade. The dose of 0.1 mg/kg i.v. pentolinium was selected since it induces a 30-min decrease in sympathetic tone; this decrease in sympathetic tone remaining constant between 10 and 20 min after pentolinium ($t0$).

Two series of experiments were performed to compare the effects of i.v. injection of NaCl (0.9%) and fenoldopam (20 μg/kg) in the barodenervated dogs treated with pentolinium (0.1 mg/kg). Each animal received fenoldopam (fenoldopam-treated group: $n=6$) or NaCl 0.9% (control group: $n=6$). The dopamine D1-like receptor agonist or NaCl 0.9% was injected 15 min after pentolinium. Systolic blood pressure and diastolic blood pressure as well as noradrenaline plasma levels were measured before pentolinium ($t-5$) and after pentolinium ($t+17$ and $t+20$), that is, respectively 2 and 5 min after fenoldopam injection.

2.2.3. Ganglionic stimulation

The dose of 20 μg/kg nicotine was defined according to the study of Jain et al. (1997) and to previous experiments on anaesthetised dogs. Two series of experiments were performed with sinoaortic denervated dogs to compare the effects of i.v. nicotine (20 μg/kg) after fenoldopam or NaCl 0.9%. In the fenoldopam-treated group ($n=6$), the dopamine D1-like receptor agonist was injected 1 min before nicotine ($t0$). In the control group ($n=6$), the same animals were injected with NaCl 0.9% instead of fenoldopam. Systolic blood pressure and diastolic blood pressure as well as noradrenaline plasma levels were measured before any injection ($t-5$) and after nicotine injection ($t+1$, $t+4$, $t+6$ and $t+10$ min).

2.3. Cardiovascular parameters, arterial blood sampling and biochemical assays

Systolic blood pressure and diastolic blood pressure and heart rate were recorded by means of a catheter introduced into the abdominal aorta via the femoral artery and connected to a Transpac IV pressure transducer (Abbott, Ireland) and to a Qazap 94104 amplificator (Bionic Instruments, France) coupled to a MacLab hardware unit (ADInstruments, MacLab/4S, Australia) connected to a microcomputer (Power MacIntosh 6200, Apple, USA).

Plasma noradrenaline was measured by high-pressure liquid chromatography, using electrochemical (amperometric) detection. Briefly, fresh blood was collected from the femoral artery on lithium heparin with sodium metabisulfite (10 M) and centrifuged (2000 × g, 10 min at 0 °C). Plasma was stored at −80 °C. Noradrenaline was selectively isolated from the sample by adsorption on activated alumina, then eluted with 0.1 M acetic acid. Dihydroxybenzylamine was used as an internal standard to monitor recovery from this extraction step. The working electrode potential was set at 0.65 V against an Ag/AgCl reference electrode. Under these conditions, the detection limit was 0.03 nM (Damase-Michel et al., 1990).

2.4. Statistical analysis

All data are presented as mean values ± S.E.M. Assuming that all experiments are independent since they were

performed at least 1 week apart, statistical analysis was performed after analysis of variance (ANOVA with repeated measures) followed by a non-parametric paired Wilcoxon's *t*-test for intragroup analysis or Mann–Whitney *U*-test for intergroup analysis. Values at the level of $P<0.05$ were considered significantly different.

2.5. Chemicals

Drugs used in this study were nicotine [L-1-methyl-2-(3 pyridyl) pyrrolidine, Sigma, St. Louis, MO, USA], a nicotinic acetylcholine receptor agonist, and pentolinium (1-1′ -pentamethylene bis [1-methyl pyrrolidinium hydrogen tartrate]), Sigma-Aldrich, St. Quentin Fallavier, France), a peripheral ganglionic blocker that does not cross the blood-brain barrier. Fenoldopam mesylate (6-chloro-2,3,4,5-tetra-(hydroxyphenyl)-1*H*-3-benzazepine-7,8-dione methane sulfonate), a dopamine D1 receptor agonist, was a gift from Smith Kline & French Laboratories, Philadelphia, PA, USA).

3. Results

3.1. Effects of fenoldopam (20 μg/kg i.v.)

Intravenous injection of NaCl (0.9%) modified neither systolic blood pressure, diastolic blood pressure, heart rate nor noradrenaline plasma levels.

Fenoldopam (20 μg/kg) induced a marked decrease in systolic blood pressure and diastolic blood pressure which

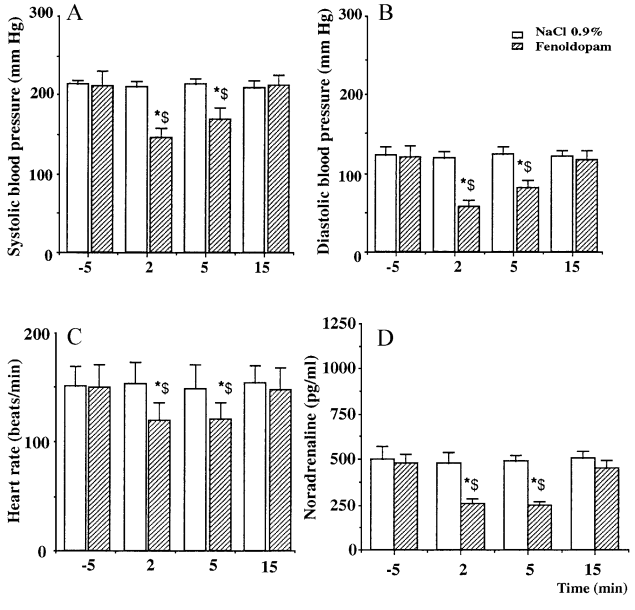

Fig. 1. Effect of NaCl 0.9% [blank column] ($n=6$) or fenoldopam (20 μg/kg i.v.) [hatched columns] ($n=8$) on systolic blood pressure (A), diastolic blood pressure (B), heart rate (C) and noradrenaline plasma levels (D) in sinoaortic denervated conscious dogs. Mean ± S.E.M., $*P<0.05$ when compared with basal values, \$ $P<0.05$ when compared with control (NaCl 0.9%) values.

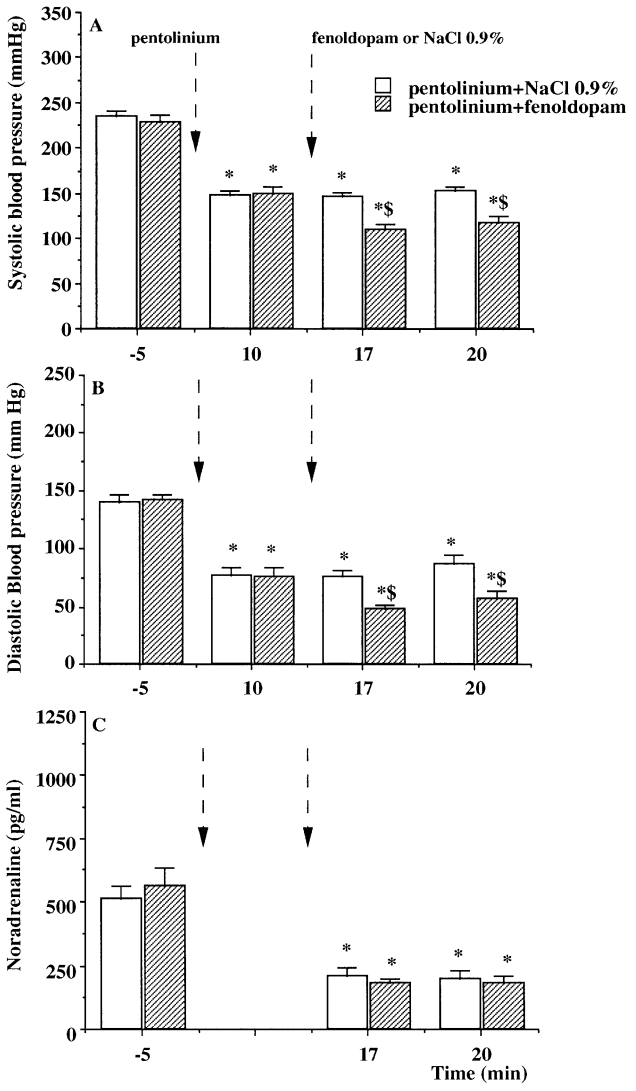

Fig. 2. Effect of i.v. injection of NaCl 0.9% ($n=6$) [blank column] or i.v. fenoldopam 20 μg/kg ($n=6$) [hatched columns] in sinoaortic denervated dogs on systolic blood pressure (A), diastolic blood pressure (B) and noradrenaline plasma levels (C) measured at $t-5$ and at $t+10$, $t+17$, $t+20$ after i.v. pentolinium injection (0.1 mg/kg). Mean ± S.E.M., $*P<0.05$ when compared with basal values, \$ $P<0.05$ when compared with control (NaCl 0.9%) values.

was significant at $t+2$ and $t+5$ versus basal values ($P<0.05$) or in the intergroup analysis comparing fenoldopam- and NaCl-treated groups ($P<0.05$). Simultaneously, we observed a decrease in heart rate which was significant at $t+2$ and $t+5$ versus basal values ($P<0.05$) or on intergroup analysis ($P<0.05$).

A significant decrease in noradrenaline plasma levels was observed at $t+2$ and $t+5$ versus basal values ($P<0.05$) or on intergroup analysis ($P<0.05$) (Fig. 1).

3.2. Effect of ganglionic blockade

Resting values ($t-5$) for systolic blood pressure and diastolic blood pressure were not significantly different

between the two groups: pentolinium + NaCl (control group) and pentolinium + fenoldopam (fenoldopam-treated group). In both groups, i.v. injection of pentolinium (0.1 mg/kg) significantly decreased systolic blood pressure and diastolic blood pressure, 10, 17 and 20 min after i.v. pentolinium, when compared to basal values ($P<0.05$). Intergroup analysis showed that fenoldopam (20 μg/kg) administration elicited a significant decrease in systolic and diastolic blood pressure, 2 and 5 min after fenoldopam injection (i.e. 17 and 20 min after pentolinium administration) when compared to the control group at $t+17$ and $t+20$ ($P<0.05$) (Fig. 2A,B).

Resting values ($t-5$) for noradrenaline plasma levels were not significantly different in control and in fenoldopam-treated groups. Intravenous pentolinium elicited a significant decrease, compared to basal values, in noradrenaline plasma levels in both groups at $t+17$ and $t+20$ ($P<0.05$). Intergroup analysis showed that fenoldopam (20 μg/kg) administration did not significantly modify noradrenaline plasma levels when compared to the control group at $t+17$ and $t+20$ (Fig. 2C).

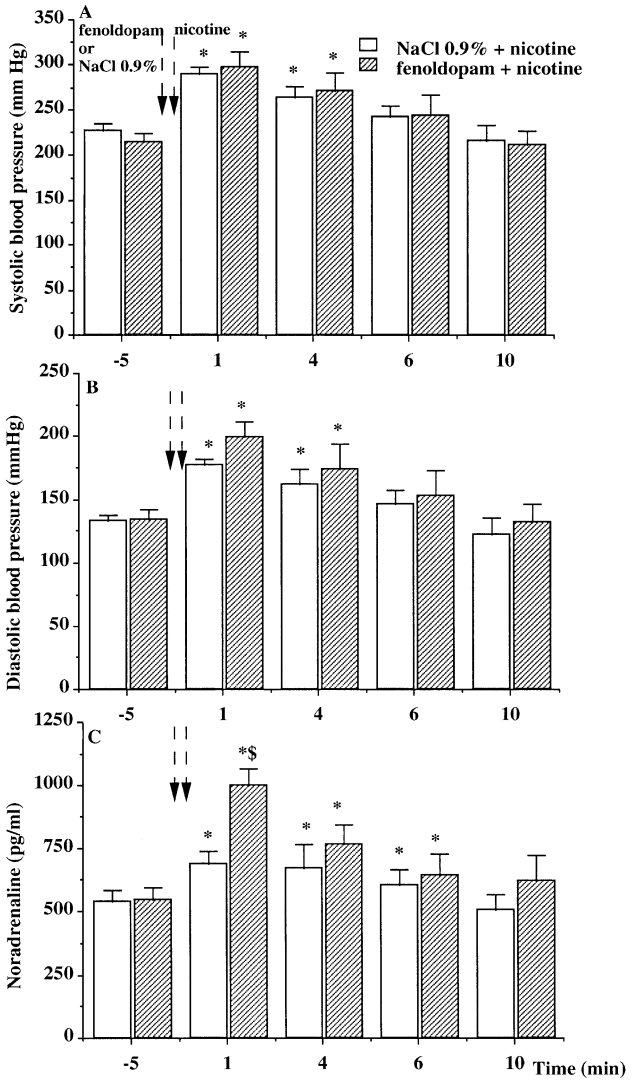

Fig. 3. Effect of i.v. injection of nicotine (20 μg/kg) after NaCl 0.9% ($n=6$) [blank column] or i.v. fenoldopam 20 μg/kg ($n=6$) [hatched columns] in sinoaortic denervated dogs on systolic blood pressure (A), diastolic blood pressure (B) and noradrenaline plasma levels (C) measured at $t-5$ and at $t+1$, $t+4$, $t+6$ and $t+10$ after i.v. nicotine injection. Mean ± S.E.M., *$P<0.05$ when compared with basal values, $ $P<0.05$ when compared with control (NaCl 0.9%) values.

3.3. Effect of ganglionic stimulation

The basal values ($t-5$) for systolic blood pressure and diastolic blood pressure did not differ significantly between the two groups [(NaCl + nicotine (control group) and fenoldopam + nicotine)].

In both groups, i.v. nicotine injection (20 μg/kg) resulted in a marked and short increase in systolic blood pressure and diastolic blood pressure, which was significant ($P<0.05$) 1 and 4 min later versus basal values.

Intergroup analysis showed that fenoldopam (20 μg/kg) administration did not significantly modify the nicotine-induced pressor effect when compared to the control group (Fig. 3A,B).

The basal values for noradrenaline plasma levels were not significantly different between the two groups [(NaCl 0.9% + nicotine (controls) and fenoldopam + nicotine)]. Moreover, in both groups, i.v. nicotine (20 μg/kg) significantly increased plasma noradrenaline levels at $t+1$, $t+4$ and $t+6$ min after nicotine injection ($P<0.05$) when compared to basal values.

Intergroup analysis showed a significant increase in noradrenaline plasma levels at $t+1$ after nicotine injection in the fenoldopam-treated group versus the control group ($P<0.05$) (Fig. 3C).

4. Discussion

The present study investigated the effects of fenoldopam, a specific dopamine D1-like receptor agonist, on cardiovascular responses and sympathetic tone in sinoaortic denervated dogs. This study showed bradycardia and a decrease in sympathetic tone after dopamine D1-like receptor stimulation in barodenervated dogs.

We investigated the mechanism involved in the decrease in sympathetic tone observed after administration of a dopamine D1-like receptor agonist in barodenervated dogs.

Two mechanisms can be proposed to explain the decrease in sympathetic tone: involvement of (1) central or of (2) peripheral pathways.

(1) Involvement of central pathways in the decrease in sympathetic tone can be excluded since previous experiments have shown that intracisternal injection of several doses of fenoldopam (1–10 μg/kg) modified neither cardiovascular parameters nor noradrenaline plasma levels in conscious dogs (Lokhandwala et al., 1985; Damase-Michel et al., 1995). Moreover, it has been suggested that fenoldo-

pam is not able to cross the blood-brain barrier (McCoy et al., 1986). Thus, the lack of central effect of fenoldopam suggests that the decrease in sympathetic tone observed in sinoaortic denervated dogs is due to a peripheral mechanism. (2) Fenoldopam could act on the efferent pathway of the sympathetic nervous system. As far as we know, dopamine D1-like receptors have never been described on presynaptic post-ganglionic nerve endings. However, as already mentioned in the Introduction, some authors have found these receptors on sympathetic ganglia (Sabouni et al., 1986; Shebuski et al., 1985) whereas others (Kimura et al., 1992; Satoh et al., 1989) failed to identify them.

These conflicting data led us to explore ganglionic transmission after fenoldopam administration, using two complementary pharmacological approaches, ganglionic blockade and stimulation.

In the experiments on ganglionic blockade, pentolinium (0.1 mg/kg) [which is a peripheral antagonist of the nicotinic receptors (Gaspo et al., 1994)] elicited a 60% decrease in noradrenaline plasma levels. After pentolinium, fenoldopam failed to induce any change in sympathetic tone when compared to the control group (Fig. 2C). However, the direct vasodilatation resulting from dopamine D1-like postsynaptic receptor stimulation always remained present, as shown by the fenoldopam-induced decrease in blood pressure observed in the intergroup analysis at $t17$ and $t20$ (Fig. 2A,B).

Thus, partial blockade of nicotinic receptors abolished the fenoldopam-induced decrease in sympathetic tone. The mechanism involved in the fenoldopam-induced decrease in sympathetic tone seems to be of ganglionic origin. This finding also suggests that dopamine D1-like receptors are absent at the postsynaptic level of the ganglion: if not, the stimulation of such a dopamine D1-like ganglionic receptor located on the postsynaptic side of the ganglion would have induced a further decrease in noradrenaline release due to partial ganglionic blockade. We can conclude that dopamine D1-like ganglionic receptors are located at the presynaptic level of the ganglion.

Stimulation of ganglionic nicotinic receptors with nicotine (20 μg/kg) elicited a rapid and significant increase in noradrenaline plasma levels. The maximal increase was observed 1 min after i.v. injection. In the fenoldopam-treated group, we never observed a decrease in noradrenaline plasma levels when compared to the control group. Thus, this result confirms the absence of a dopamine D1-like receptor at the postsynaptic level of the ganglia, the stimulation of which would have resulted in a decrease in noradrenaline release. Thus, the dopamine D1-like receptor is located at the presynaptic level of the ganglia. Its stimulation induced a decrease in acetylcholine release. These "in vivo" results are in accordance with the "in vitro" results of Mukai et al. (1996), suggesting that presynaptic dopamine D1-like receptors exert an inhibitory effect on acetylcholine release in isolated dog cardiac sympathetic ganglia (Fig. 4).

Concerning experiments with ganglionic blockade and stimulation, two points need further comment: (1) Under the partial ganglionic blockade obtained after 0.1 mg/kg pentolinium, fenoldopam-induced dopamine D1-like receptor stimulation failed to further decrease noradrenaline plasma levels. One can hypothesise that, after pentolinium partial blockade, the number of remaining free nicotinic receptors was low so that reduced acetylcholine release (after fenoldopam) was still sufficient to stimulate them. (2) Concerning experiments with ganglionic stimulation, our results show a significant increase in noradrenaline plasma levels 1 min after nicotine injection in the fenoldopam-treated group when compared to the control group. The existence of a dopamine D1-like receptor with facilitating effects cannot be assumed since, if such a receptor existed, we

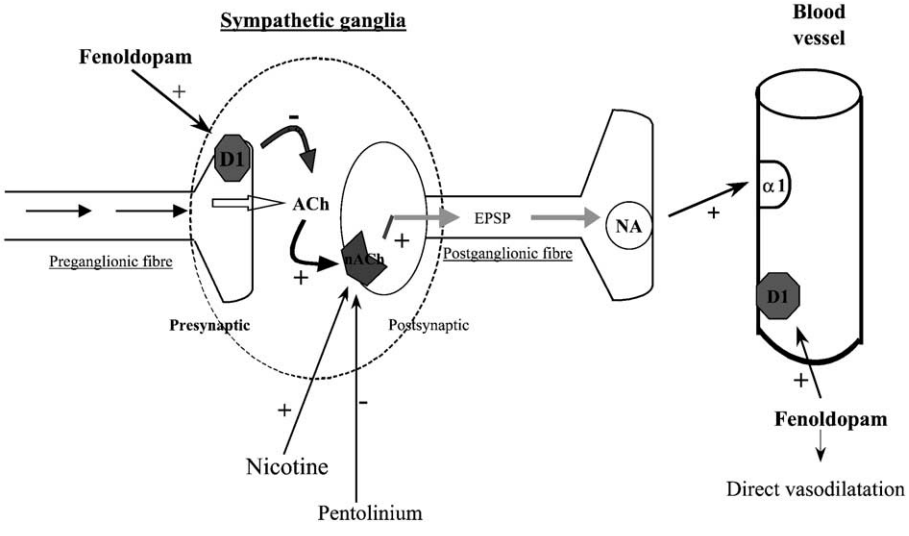

Fig. 4. Schematic drawing of ganglionic transmission pathways showing the putative involvement of dopamine D1-like receptors in the regulation of sympathetic tone. D1, dopamine D1-like receptor; nAch, nicotinic receptor; Ach, acetylcholine; EPSP, excitatory postsynaptic potential; NA, noradrenaline; α1, alpha-adrenergic receptor.

would never have observed a decrease in sympathetic tone after dopamine D1 receptor agonist administration in barodenervated dogs (Fig. 1). This effect could be explained as follows: in the fenoldopam-treated group, the dopamine D1 receptor agonist decreases acetylcholine release when compared to the control group. One can hypothesise that the greater release of noradrenaline can be explained by a greater intrinsic activity of nicotine (when compared to acetylcholine) on free nicotinic receptors. In contrast, fenoldopam administration did not significantly modify the nicotine-induced pressor effect. One can suggest that the direct vasodilating effect of fenoldopam on vascular smooth muscle could balance the vasopressor action induced by the increase in sympathetic tone.

Thus, two types of dopamine peripheral receptors (dopamine-D1 and dopamine-D2) are present at the ganglionic level, their stimulation leading to a decrease in sympathetic tone. The physiological role of these dopamine receptors in the modulation of ganglionic transmission remains to be determined. Nevertheless, one can suggest that this sympatholytic property could participate in the antihypertensive effects of dopamine D1 receptor agonists, already used for their vasodilating and natriuretic properties.

References

Damase-Michel, C., Valet, P., Montastruc, J.L., 1987. Nicardipine causes sympathetic activation that does not involve baroreflex tachycardia in conscious sino-aortic-denervated dogs. Eur. J. Pharmacol. 42, 145–149.

Damase-Michel, C., Tran, M.A., Montastruc, J.L., Moatti, J.P., Montastruc, P., 1989. Effects of pinacidil on the sympathoadrenal system in dogs. Br. J. Pharmacol. 97, 1019–1026.

Damase-Michel, C., Montastruc, J.L., Gharib, C., Geelen, G., De Saint-Blanquat, G., Tran, M.A., 1990. Effect of quinpirole, a specific dopamine DA2 receptor agonist on the sympathoadrenal system in dogs. J. Pharmacol. Exp. Ther. 252, 770–777.

Damase-Michel, C., Montastruc, J.L., Tran, M.A., 1995. Effects of dopaminergic drugs on the sympathoadrenal system. Hypertens. Res. 18, S119–S124.

Gaspo, R., Yamaguchi, N., De Champlain, J., 1994. Nifedipine inhibits adrenal but not circulating catecholamine response to nicotinic stimulation in dogs. Am. J. Physiol. 267, R1545–R1551.

Jain, A.C., Mehta, M.C., Billie, M., 1997. Combined effects of caffeine and nicotine on cardiovascular hemodynamics in canine model. J. Cardiovasc. Pharmacol. 29, 574–579.

Jose, P.A., Eisner, G.M., Felder, R.A., 2002. Role of dopamine receptors in the kidney in the regulation of blood pressure. Curr. Opin. Nephrol. Hypertens. 11, 87–92.

Kimura, T., Mukaiyama, O., Satoh, M., 1992. Effects of dopamine and α2-adrenoceptor agonist and antagonist on muscarinic receptors stimulation in cardiac sympathetic ganglia of the dog. J. Cardiovasc. Pharmacol. 19, 382–386.

Kohli, J.D., Glock, D., Goldberg, L.I., 1988. Relative DA1-dopamine-receptor agonist and alpha-adrenoceptor antagonist activity of fenoldopam in the anesthetized dog. J. Cardiovasc. Pharmacol. 11, 123–126.

Lokhandwala, M.F., Watkins, H.O., Sabouni, M.H., Alkhadi, K.A., 1985. Pharmacological analysis of the actions of SKF 82526 on cardiovascular dopamine receptors. J. Pharmacol. Exp. Ther. 234, 337–343.

McCoy, C.E., Douglas, F.L., Goldberg, L.I., 1986. Selective antagonism of the hypotensive effects of dopamine agonists in spontaneously hypertensive rats. Hypertension 8, 298–302.

Mukai, M., Kushiku, K., Yamada, H., Yamada, K., Dan, K., Furukawa, T., 1996. The inhibition of ganglionic transmission via presynaptic dopamine DA1 and postsynaptic DA2 receptor activation in the canine cardiac sympathetic ganglia. J. Pharmacol. Exp. Ther. 279, 822–829.

Nichols, A.J., Ruffolo, R.R., Brooks, D.P., 1990. The pharmacology of fenoldopam. Am. J. Hypertens. 3, 116S–119S.

Sabouni, M.H., Alkadhi, K.A., Lokhandwala, M.F., 1986. Pharmacological characterization of dopamine receptors in the stellate ganglia with selective DA1 and DA2 receptor agonists and antagonists. J. Pharmacol. Exp. Ther. 236, 65–70.

Satoh, Y., Kohli, J.D., Goldberg, L.I., 1989. Effects of alpha-adrenoceptor and dopamine receptor agonists and antagonists on ganglionic transmission. J. Pharmacol. Exp. Ther. 251, 253–257.

Shebuski, R.J., Fulita, T., Smith, J.M., Kopaciewicz, L.J., Blumberg, A.L., Hieble, J.P., 1985. Suppression of sympathetic neurotransmission by selective dopamine-1 receptor agonist fenoldopam (SKF82526) in anesthetized dog. J. Pharmacol. Exp. Ther. 235, 735–740.