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Inhibitory Effects of Dopamine on Noradrenaline-induced Constriction of Arterioles *in vivo* in the Striated Cremaster Muscle

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ABSTRACT

The effect of dopamine on the arterioles (50.8~95.2 μm) in the cremaster muscle was examined to determine its effect on microcirculation. Anesthetized rats were used under a light microscope connected to a videocamera. Drugs were applied using small round filter paper (370 μm in diameter) containing the drug and placed in the immediate vicinity of the arteriole on the cremaster with a micromanipulator. The dose of the drug applied was represented by concentration of the drug solution in which the filter paper was immersed. Dopamine (10^{-6}~10^{-4}M) induced neither constriction nor dilation of the arteriole in the cremaster. Papaverine (10^{-2}M) did not dilate the arteriole. However, the arterioles were constricted by noradrenaline (10^{-6}~10^{-4}M) and vasopressin (10^{-7}M) in a dose-dependent manner. Noradrenaline (10^{-4}M)-induced constriction was blocked by concomitant application of dopamine (10^{-4}M). This effect of dopamine was antagonized by SCH23390 (10^{-3}M). However, isoproterenol (10^{-3}M) did not affect the arteriole, nor inhibit noradrenaline (10^{-4}M)-induced constriction of the arterioles. While forskolin (10^{-2}M) alone did not produce constriction or dilation of the arterioles, it inhibited noradrenaline (10^{-4}M)-induced constriction of the arteriole. These results suggest that dopamine prevents the constriction of the arteriole induced by noradrenaline, by activation of DA_1 receptors, which activates adenylate cyclase.

Key words: *Dopamine, Noradrenaline-induced constriction, Forskolin, Arteriole in rat cremaster*

Dopamine is an important neurotransmitter in the nigrostriatal, mesocorticolimbic and tuberohypophysial systems, which are involved in movement, emotional-cognitive and endocrine functions, respectively. This substance is also located in ganglions and in the gastrointestinal tract to regulate ganglionic transmission and bowel motility, respectively[17]. In addition, the richly distributed dopamine in the renal cortex acts on the renal tubules to produce a natriuretic action[16]. More importantly, Goldberg and his collaborators found that peripheral dopamine receptors located on the smooth muscle of renal and mesenteric arteries induced vasodilation when activated[4,15]. Cardiac action of dopamine has also been documented[4,10]. Based on these discoveries, dopamine has been clinically used for the treatment of shock and congestive heart failures[5-7,13], because of its vasodilating effects on the renal artery and cardiac action via the DA_1 and β_1 receptors, respectively[5,6,12].

Noradrenaline and adrenaline play dominant roles in the cardiovascular system so that arteries are constricted and dilated via α_1 and β_2 receptors, respectively[9]. In a similar manner, dopamine also participates in the regulation of vascular tone; it induces vasodilation of renal, mesenteric, basilar, coronary, subcutaneous, omental and splenic arteries[2,3-8,21]. This vasodilation by dopamine is mediated by the DA_1 receptor, whose activation elevates the cyclic-AMP level[2,18].

Since the 15~20% and 80~85% of cardiac outputs in the resting and working states, respectively are responsible for the blood flow in striated muscles[19], regulation of arteries in such tissues (especially the arterioles) is physiologically important. However, effects of dopamine on arterioles in the striated muscle remain hitherto unknown. Therefore, the present *in vivo* study attempted to elucidate whether or not dopamine participated in the regulation of arterioles in the striated muscle of anesthetized rats.

MATERIALS AND METHODS

Animals

Male wistar rats (aged 7~12 weeks) weighing 250~400g were anesthetized with sodium pentobarbital (60mg/kg, i.p.). Additional sodium pentobarbital (20 mg/kg) was given intraperitoneally

every 45～60 min to maintain the anesthesia.

Preparation and Instruments

The cremaster preparation was made by a modification of Majno's method[14]. Briefly, a white spatula was inserted beneath the cremaster muscle from the fistula, in the abdominal wall, in order to hold the cremaster flat and to improve optical conditions. The microcirculation in the cremaster was observed by light optic microscopy (NIKON, 2×5). The microscopic image was magnified on a videomonitor (SHARP, CZ–802D) through a videocamera (VICTOR, GX–N7). The image was simultaneously recorded on videotape using a videocassette recorder (SONY, SL–HF900MK II).

The cremaster was illuminated bilaterally at 45° to the horizontal plane using two fiber optic light sources (NIKON, SMZ–10). This illumination produced an extremely bright visual field with a small amount of heat and prevented drying of the cremaster. The optimal exposure was adjusted by controlling the amount of light with an exposure meter in the videocamera.

After experiments, the videotape was replayed, and the images on the video monitor were printed out as graphic images by video graphic printer (SONY, UP-811). The diameter of the arterioles was measured using the scale loupe (PEAK ×10) on the graphic image.

Application of the drugs

Small round filter papers immersed in drug solutions were placed on the cremaster, in the immediate vicinity of the arteriole, and monitored by videomonitor image with a micromanipulator. The small filter paper (370 μm in diameter) was made by punching out Whatman filter paper (No. 40) with a puncher specially made with an 1/1 subcutaneous injection needle. A small filter paper was immersed in water-soluble ink (Recorder Ink, Nihon Kohden) and placed in the immediate vicinity of the arteriole on the cremaster. The ink spread radially and covered the arteriole in 2～3 min, but remained within the visual field on the videomonitor image (Fig. 1). The dose of the drug applied was represented by concentration of the drug solution, in which the filter paper was immersed. The exact concentration of drug on the arteriole could not be determined. However, the dose applied can be derived from the concentra-

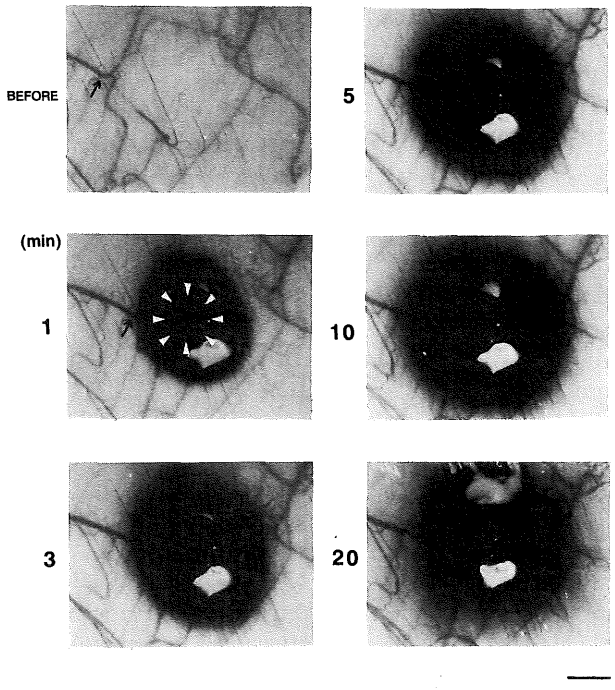

Fig. 1. Diffusion of black ink from the small filter paper (indicated by white triangles), immersed in ink, and placed in the immediate vicinity of the arteriole indicated by arrow. Graphic images are shown before, and 1, 3, 5, 10, and 20 min after placement of the filter paper containing ink. The ink spread radially around the filter paper until 10 min after application. After 10 min, however, the ink remained within the visual field on the videomonitor image. The horizontal bar: 500 μm, Numbers on the left: min after application of black ink.

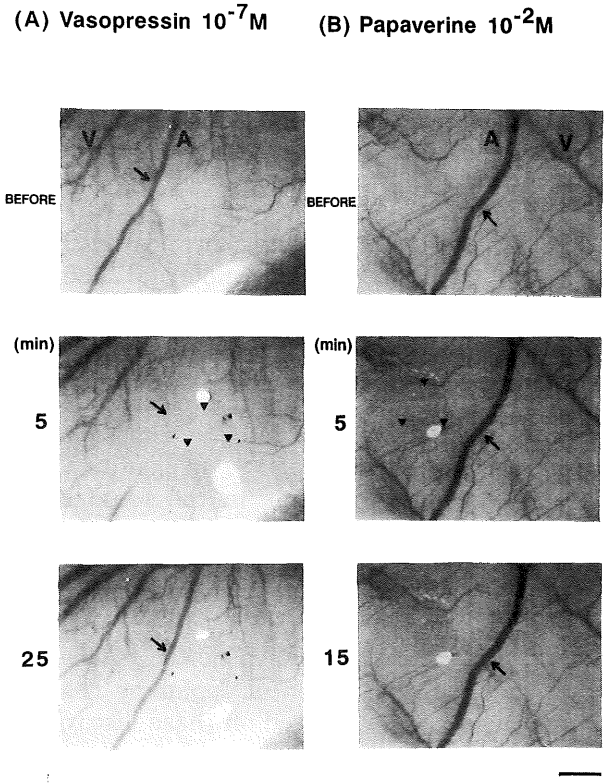

Fig. 2. Effects of vasopressin 10^{-7}M (A) and papaverine 10^{-2}M (B) on the arteriole. Arrows indicate the arteriole under observation. Closed triangles indicate the filter paper containing vasopressin or papaverine. The horizontal bar: 500 μm, A: arteriole, V: venule, Numbers on the left: min after application of drugs.

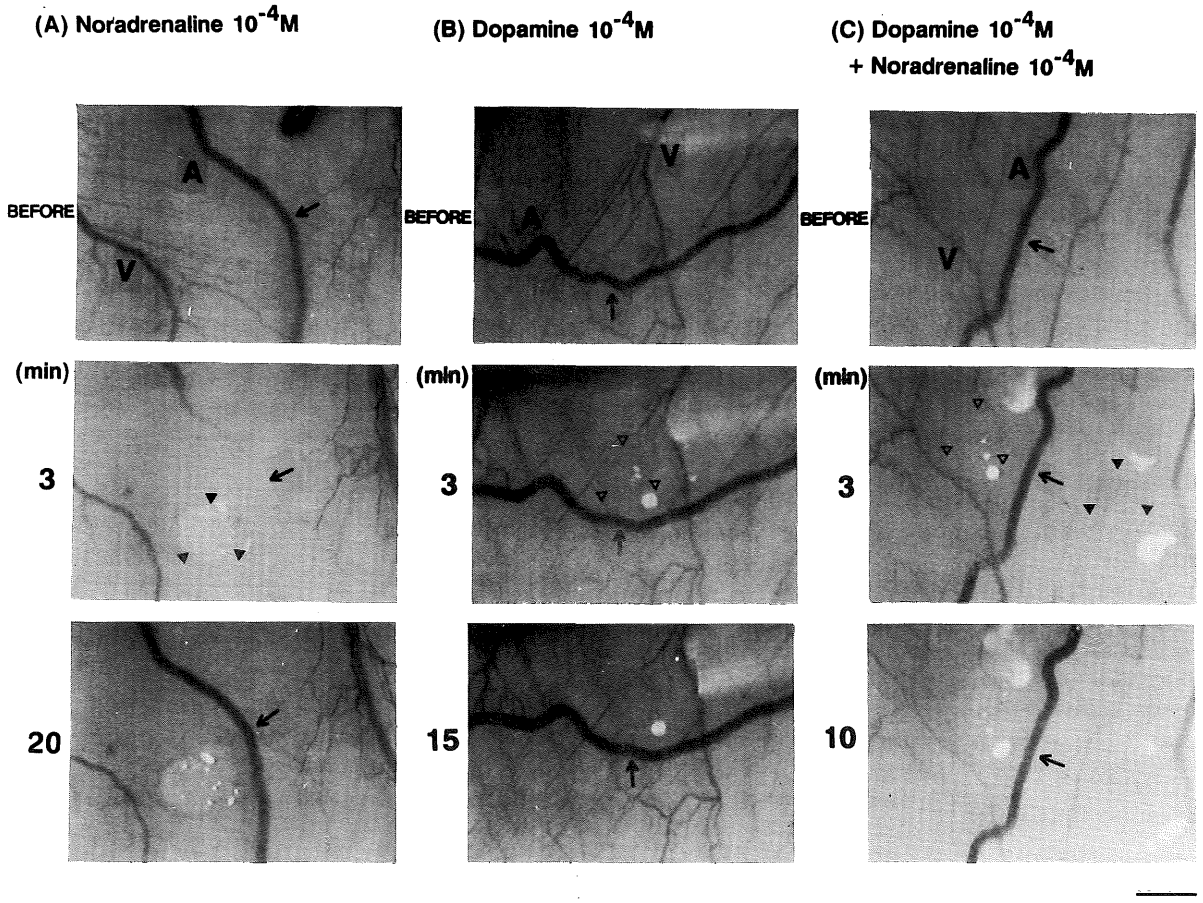

Fig. 3. Effects of noradrenaline (A) and dopamine (B) on the arteriole, and inhibition by dopamine on noradrenaline-induced constriction of the arteriole (C). Arrows show the arterioles under observation. Closed and Open triangles indicate the filter paper containing noradrenaline and dopamine, respectively. Noradrenaline (10^{-4}M) was applied 5 min after the application of 10^{-4}M dopamine. The horizontal bar: 500 μm A: arteriole, V: venule, Numbers on the left: min after application of drugs.

tion of drug solution in which the filter paper was immersed. The total amount of the drug in the filter paper was very small and the topically applied drug distributed extensively to be eventually diluted with tissue fluid. If the dispersion of drug in the filter paper (370 μm in diameter) was in a manner similar to black ink, the diameter of the dispersion was approximated to 2100 μm in 5 min and the concentration in the arteriole was estimated to be at most 100 times lower than that in the immersed paper. Thus, concentrations around the arterioles of noradrenaline, dopamine, isoproterenol, SCH23390, vasopressin, papaverine and forskolin are considered to be at most 100 times lower than those in the filter paper. Therefore, the concentrations of these drugs used in this experiment are not much different from those used to induce responses of *in vitro* experiments in the bath[18,20,22,23]. The cremaster, including the arteriole, was washed with approximately 5 ml of Tyrode solution, after removal of the drug-immersed paper, and another arteriole was used for the next experiment.

Drugs

The drugs used were: noradrenaline hydrochloride (Sigma), dopamine hydrochloride (Katayama chemical), isoproterenol hydrochloride (Sigma), SCH23390 hydrochloride (Research Biochemicals), forskolin hydrochloride (Research Biochemicals), vasopressin (Sigma), and papaverine hydrochloride (Tokyo Chemicals). Each drug was dissolved in Tyrode solution, composed of (in mM): NaCl 137, KCl 2.7, $CaCl_2$ 1.8, $MgCl_2$ 0.5, NaH_2PO_4 0.4, $NaHCO_3$ 1.19, and glucose 5.6. Statistical significance was determined using the non-paired t test.

RESULTS

The arterioles ranging from 50.8~95.2 μm in diameter (mean ± S.E. = 77.7 ± 1.3 μm, n=72) were used for the experiment.

1. Effects of noradrenaline, vasopressin, and papaverine

The arteriole was constricted by 10^{-7}M vasopressin (Fig. 2–A). The maximum constriction

with vasopressin was seen 3～10 min after application, and the arteriole returned to its original diameter 25～30 min later. The arteriole was also constricted by noradrenaline (10^{-6}～10^{-4}M) in a dose-dependent manner (Fig. 3–A and Fig. 4). When 10^{-4}M noradrenaline was applied, the diameter of the arteriole decreased to 3.7 ± 1.7 μm (n=6) (Table 1). Maximum constriction was obtained with noradrenaline (10^{-4}M) 2～3 min after the application, with recovery seen 20 min later (Fig. 3–A). However, the arteriole was not dilated by 10^{-2}M papaverine (Fig. 2–B). Dilation or constriction of the arteriole was not induced by dopamine at doses up to 10^{-4}M (Fig. 3–B and Fig. 4).

Table 1. Effects of vasoconstrictive and vasodilative drugs and their antagonists on the diameter of the arteriole.

(M)	n	Diameter of arteriole at maximal constriction (mean ± S.E.) (μm)	% of control (mean ± S.E.)
Initial Diameter	72	77.7 ± 1.3	100.0
Noradrenaline (10^{-4})	6	3.7 ± 1.7	4.0 ± 1.9
Dopamine (10^{-4})	8	73.8 ± 4.0	100.0 ± 0.0
Dopamine (10^{-4}) + Noradrenaline (10^{-4})	8	68.3 ± 6.1	* 91.6 ± 4.9
SCH23390 (10^{-3})	8	68.3 ± 3.3	100.0 ± 0.0
SCH23390 (10^{-3}) + Dopamine (10^{-4}) + Noradrenaline (10^{-4})	8	4.4 ± 2.2	6.5 ± 3.2 #
Forskolin (10^{-2})	5	74.9 ± 2.6	100.0 ± 0.0
Forskolin (10^{-2}) + Noradrenaline (10^{-4})	5	73.0 ± 3.5	* 97.3 ± 1.8
Isoproterenol (10^{-3})	7	67.1 ± 4.1	100.0 ± 0.0
Isoproterenol (10^{-3}) + Noradrenaline (10^{-4})	7	3.2 ± 1.7	4.5 ± 2.2 #
Vasopressin (10^{-7})	6	0.5 ± 0.5	0.6 ± 0.6
Papaverine (10^{-2})	4	85.7 ± 4.1	100.0 ± 0.0

Values are expressed as %, and 100% is taken as the value before application of the drugs. The diameter of the arteriole, maximally constricted, was measured 2～3 min after application of noradrenaline (10^{-4}M), in the absence and presence of other drugs, and 3～10 min after application of vasopressin (10^{-7}M). The effects of dopamine, SCH23390, forskolin, isoproterenol, and papaverine were taken 5 min after the application of each drug alone. *: $p<0.01$, significantly different from noradrenaline (10^{-4}M) alone. #: $p<0.01$, significantly different from noradrenaline (10^{-4}M) in the presence of dopamine (10^{-4}M).

2. Effect of dopamine on noradrenaline-induced constriction of the arteriole

Dopamine alone at doses up to 10^{-4}M did not produce dilation or constriction of the arterioles (Fig. 3–B and Fig. 4). However, when dopamine(10^{-4}M) was applied 5 min before application of noradrenaline (10^{-4}M), the noradrenaline-induced constriction was completely blocked (Fig. 3–C). The diameter of the arteriole 3 min after application of noradrenaline (10^{-4}M), in the presence of dopamine (10^{-4}M), was significantly ($p<0.01$) greater than when induced by the same dose of noradrenaline alone. The diameters were 68.3 ± 6.1 (n=8) and 3.7 ± 1.7 (n=6) μm with noradrenaline in the presence and absence of dopamine, respectively (Table 1). When SCH23390 (10^{-3}M), a D_1 antagonist, was applied 5 min before the application of 10^{-4}M dopamine, and then noradrenaline (10^{-4}M) was applied 5 min later, the diameter of the arteriole was significantly decreased, compared with that in the absence of the D_1 antagonist (Fig. 5 and Table 1).

3. Effect of isoproterenol on noradrenaline-induced constriction of the arteriole

Isoproterenol (10^{-3}M) alone did not dilate or constrict the arteriole in the cremaster. Isoproterenol (10^{-3}M) was applied 5 min before 10^{-4}M noradrenaline. Furthermore, noradrenaline-induced constriction of the arteriole was not altered by pretreatment with isoproterenol (Fig. 6 and Table 1).

4. Effect of forskolin on noradrenaline-induced constriction of the arteriole

The local application of forskolin (10^{-2}M) induced neither constriction nor dilation of the arteriole. However, the application of noradrenaline (10^{-4}M) 5 min after the application of forskolin (10^{-2}M), did not induce constriction of the arteri-

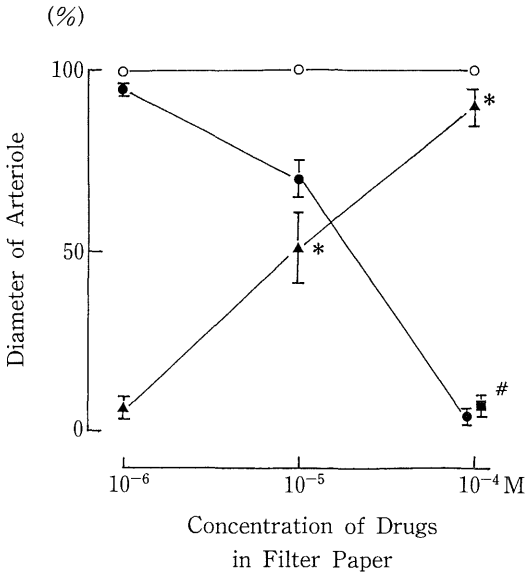

Fig. 4. Antagonizing effects of SCH23390 on inhibition by dopamine of noradrenaline-induced constriction of the arteriole. The diameter of the arteriole was taken as 100% before the application of drugs. The diameter of the arteriole, maximally constricted, was measured at 2～3 min after application of noradrenaline alone, noradrenaline (10^{-4}M) plus dopamine, and noradrenaline (10^{-4}M) in presence of both dopamine (10^{-4}M) and SCH23390 (10^{-3}M). The effects of dopamine alone were taken 5 min after the application. Values represent mean ± S.E. of the arteriole diameter. ●: noradrenaline alone (10^{-6}M n=7, 10^{-5}M n=6, 10^{-4}M n=6), ▲: noradrenaline (10^{-4}M) plus dopamine (10^{-6}M n=6, 10^{-5}M n=9, 10^{-4}M n=8), ○: dopamine alone (10^{-6}M n=6, 10^{-5}M n=9, 10^{-4}M n=8), ■: noradrenaline (10^{-4}M) in presence of both dopamine (10^{-4}M) and SCH23390 (10^{-3}M), respectively. *: $p<0.01$, significantly different from the value of noradrenaline (10^{-4}M) alone. #:$p<0.01$, significantly different from the value of noradrenaline (10^{-4}M) plus dopamine (10^{-4}M).

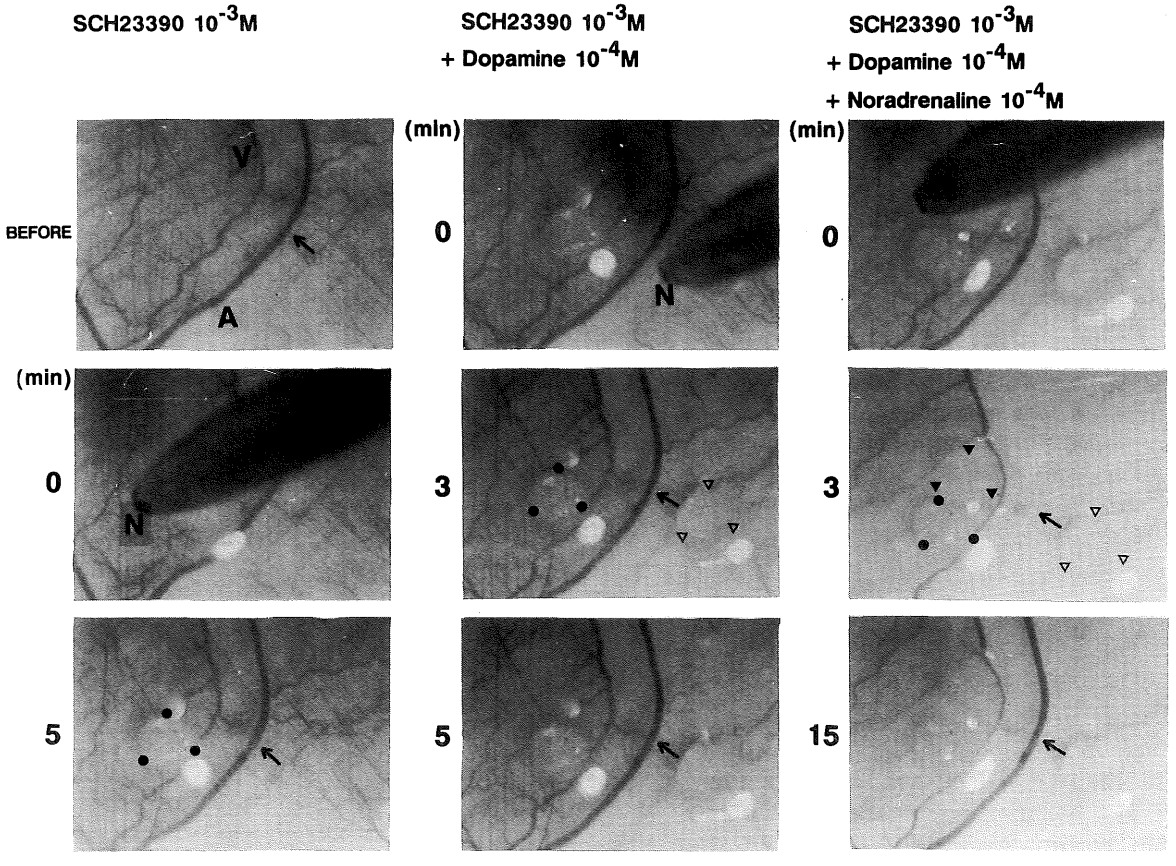

Fig. 5. Antagonizing effects of SCH23390 on inhibition by dopamine of noradrenaline-induced constriction of the arteriole. SCH23390 (10^{-3}M) was applied 5 min before application of dopamine. Dopamine (10^{-4}M) was applied 5 min after the application of SCH23390. Noradrenaline (10^{-4}M) was applied 5 min after application of 10^{-4}M dopamine. Arrows show the arteriole under observation. Closed circles, open triangles, and closed triangles indicate the filter papers containing SCH23390, dopamine, and noradrenaline, respectively. N represents the tip of the needle used for the drug application. The horizontal bar: 500 μm, A: arteriole, V: venule, Numbers on the left: min after application of drugs.

ole. The diameter of the arteriole during the application of noradrenaline (10^{-4}M), in the presence of forskolin (10^{-2}M), was significantly greater than with noradrenaline (10^{-4}M) alone (Table 1).

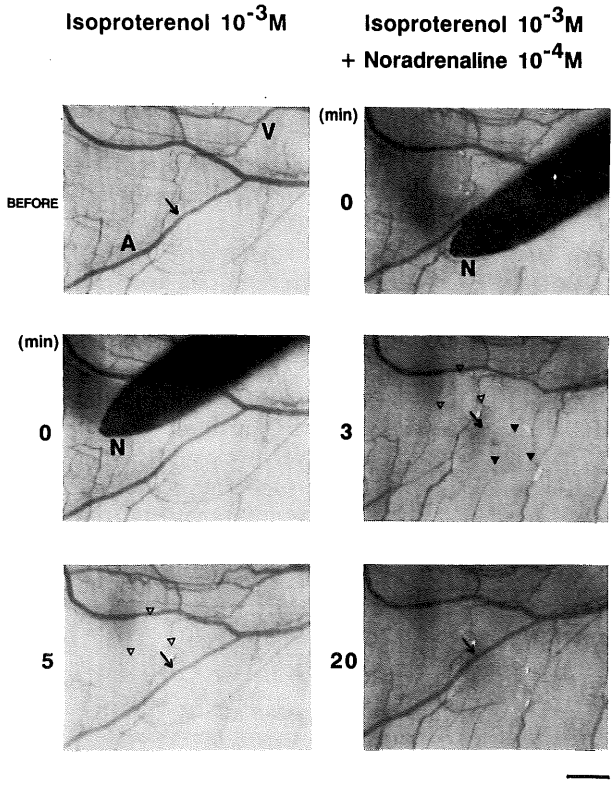

Fig. 6. Effects of isoproterenol on arteriole and noradrenaline-induced constriction of the arteriole. Isoproterenol (10^{-3}M) was applied 5 min before the application of noradrenaline (10^{-4}M). Arrows show the arteriole under observation. Open triangles and closed triangles indicate the filter papers containing isoproterenol and noradrenaline, respectively. N represents the tip of the needle used for the drug application. The horizontal bar: 500 μm, A: arteriole, V: venule, Numbers on the left: min after application of drugs.

DISCUSSION

Arterioles distributed in the cremaster muscle were constricted by noradrenaline ($10^{-6}\sim10^{-4}$M) immersed in the small filter paper in a dose-dependent manner. Black ink, when applied in a similar manner as the drugs, spread to cover the arteriole within 2~3 min, indicating that the drug reaches the arteriole under observation and acts on it. The potency of noradrenaline to constrict arterioles in the striated muscle was similar to that seen in the isolated preparation of arteries *in vitro*[23]. In addition, the arterioles with a diameter of 50.8~95.2 μm were also constricted by vasopressin at a dose equivalent to induce constriction of arteries *in vitro*[23]. Therefore, the arterioles were thought to be constricted via adrenergic α_1 and vasopressin receptors. However, our arterioles were not dilated by papaverine at a dose of 10^{-2}M, which would fully dilate the arteries[20]. This suggests that arterioles in the living cremaster muscle were fully dilated. Unlike dilation of the renal artery, the arterioles were not dilated by dopamine up to 10^{-4}M and no futher dilation was detected. These results suggest that the drugs cannot induce further dilation of arterioles in living cremaster muscle.

Noradrenaline-induced constriction was blocked by dopamine in a dose-dependent manner, and this blockade was antagonized by SCH23390, a dopamine receptor D_1 antagonist. It is not yet determined, histochemically or biochemically, whether or not dopaminergic receptors are located in arterioles that are distributed in striated muscle of the cremaster. However, our pharmacological findings suggest that dopamine acts on dopamine DA_1 receptors and increases cyclic-AMP formation, thereby relaxing the arteriole muscle constricted by noradrenaline via an α_1 adrenergic mechanism. This conclusion is also supported by our findings that forskolin, which directly activates adenylate cyclase, also inhibited noradrenaline-induced constriction of the arteriole.

In contrast to the action of dopamine, isoproterenol had no effect on the non-treated arteriole or noradrenaline-induced constriction of the arteriole. These results suggest that the arteriole is devoid of adrenaline β receptors. Therefore, the arteriole is considered to be physiologically controlled by dopamine derived from sympathetic nerve terminals[1,11] and the adrenal medulla via DA_1 receptors in such a way that, when extremely constricted by drugs such as noradrenaline, the constriction is restored by dopamine.

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