Responses of the Canine Saphenous Vascular Bed to Various Biogenic and Foreign Substances

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YAMAMOTO, M., SASAKI, H., TAIRA, N. and HASHIMOTO, K. *Responses of the Canine Saphenous Vascular Bed to Various Biogenic and Foreign Substances*. Tohoku J. exp. Med., 1970, 101 (4), 299-309 — Responses of the dog saphenous vascular bed to a variety of vasoactive substances were studied under constant flow perfusion. Adrenergic compounds, except isoproterenol, induced a constriction. Releasers of norepinephrine from the sympathetic nerve endings, such as DMPP, tolazoline, TEA and guanethidine also constricted the saphenous vascular bed, and catecholamine-uptake blocking agents such as cocaine and desipramine behaved in the same way. Phentolamine, one of the adrenergic blocking agents, and bretylium, a typical antireleaser of norepinephrine, caused a dilatation. Cholinergic compounds produced a dilatation. Serotonin, angiotensin and vasopressin produced a constriction, while histamine, bradykinin, kallikrein and edoisin produced a dilatation. Relaxants of the vascular smooth muscle, nitroglycerin, papaverine, dipyridamole and xanthine derivatives dilated the saphenous vascular bed. Ergot alkaloids caused a constriction. Morphine markedly dilated the saphenous vascular bed and nicotinic acid had no activity at all. ——— vasoactive substance; adrenergic compound; isoproterenol; catecholamine; cholinergic compound

Previously, Hashimoto and Kumakura¹ compared the vascular responses to various substances among the coronary, mesenteric, renal and femoral arteries of the dogs, and Matsumura *et al.*² extended such a comparative study further to the canine vesical vasculature. The two studies demonstrated that each peripheral artery responded differently to various substances administered intra-arterially.

The responsiveness of the blood vessels in the skin of the dog hindlimb to catecholamines and alpha-adrenergic blocking agents has been studied³ but little information is available concerning the responses to other vasoactive substances. The present study was attempted to investigate the responses to various drugs of the saphenous vascular bed of the dog perfused at constant flow.

Received for publication, March 4, 1970.

This study was partly supported by a grant from the Pharmacology Research Foundation, Inc., Tokyo.

METHODS

Twenty-one mongrel dogs of either sex weighing 8 to 15 kg were anesthetized with 30 mg/kg of sodium pentobarbital. The experimental setup is shown in Fig. 1. The right saphenous artery was exposed at the site where it runs across the surface of the gracilis muscle. A polyethylene cannula was inserted into the distal cut end of the saphenous artery. Arterial blood led from the left femoral artery was pumped into the cannulated right saphenous artery through plastic tubing by means of a kinetic clamp pump (Sigmamotor, AL-4). The shunt circuit to the left femoral vein was set up to observe if there was retrograde blood flow. An electromagnetic flowmeter (Nihon Kohden, MF-2) was arranged between the cannula in the right saphenous artery and the kinetic clamp

Fig. 1. Diagram of the perfusion system of the saphenous vascular bed.

pump. Perfusion pressure was picked up by a pressure transducer (Nihon Kohden, MP-4T) and recorded on an inkwriting oscillograph (Nihon Kohden, WI-205R).

Systemic blood pressure was measured at the carotid artery by an electromanometer (Nihon Kohden, MP-4). In each experiment the speed of the pump was initially adjusted so that perfusion pressure approximated to mean systemic blood pressure, and was kept constant throughout the experiment. Under these conditions the flow rate was 5 to 10 ml/min. In the following sections, a vasoconstriction refers to an increase and a vasodilatation to a decrease in perfusion pressure.

Experiments were usually performed under spontaneous respiration, but a respirator (Harvard Apparatus, 607) was occasionally used. Skin temperature of the perfused area was measured by a thermistor (Toa Electronics) and recorded on a polyrecorder (Toa Electronics, EPR-2T). When room temperature was maintained at 21° to 23°C and rectal temperature was controlled at 38° to 39°C by a heating device, the temperature of the perfused area ranged from 31° to 33°C.

Locke's solution, sodium ω -heparin⁴ and additional doses of sodium pentobarbital were administered through the cannulated jugular vein. Five hundred units per kg of sodium ω -heparin (supplied from Taiyo Fishery) were given just before cannulation and 1,000 U were added at one hour intervals. In order to maintain the anesthetic level, supplementary 3 to 4 mg/kg of sodium pentobarbital were added at 1 or 2 hour intervals.

TABLE 1. *Ranges of doses of investigated drugs*

Groups	Compounds	Ranges of doses (μ g)
Adrenergic drugs	Dopamine	0.1 — 30
	Norepinephrine	0.001— 1
	Epinephrine	0.001— 1
	Isoproterenol	0.03 — 0.3
	Phenylephrine	0.3 — 3
	Tyramine	1 — 30
	Methamphetamine	1 — 3
	Ephedrine	0.01 — 10
	Methoxamine	1 — 10
	Acetylcholine	1 — 3
Cholinergic drugs, and ganglion stimulating and blocking agents	Bethanechol	1 — 10
	Carbachol	0.3 — 1
	Methacholine	1 — 3
	McN-A-343	1 —100
	Nicotine	10 —100
	Lobeline	1 —300
	TMA	10 — 30
	DMPP	1 —100
	TEA	0.1 — 10*
	Serotonin	0.01 — 3
Other principal biogenic amines and active polypeptides	Histamine	1 — 3
	Angiotensin	0.1 — 3
	Vasopressin	0.1 — 0.3
	Oxytocin	0.1 — 0.3
	Bradykinin	0.01 — 0.1
	Kallikrein	0.01 — 0.03†
	Eledoisin	0.01 — 0.1
Nucleosides and nucleotides	ATP	30 —100
	ADP	30 —100
	AMP	10 —300
	Adenosine	1 —100
	TPN	100 —300
	DPN	100 —300
	CTP	100 —300
	CDP	100 —300
	CMP	100 —300
	Cytidine	100 —300
	UTP	10 —300
	UDP	100 —300
	UMP	100 —300
	Uridine	100 —300
	Nitroglycerin	0.01 — 10
Unspecific smooth muscle relaxants, analgesics and hypotensive drugs	Nicotinic acid	1 —300
	Papaverine	10 —100
	Verapamil	10 — 30
	Dipyridamole	1 —100
	Theophylline	1 —300
	Theobromine	0.1 —300
	Caffeine	1 —300
	Procaine	0.1 —3*
	Morphine	10 —100
	Hydralazine	10 —300
	Phentolamine	1 — 30
	Phenoxybenzamine	1 — 30
	Tolazoline	1 —100
	Hydergine	3 — 10
	Ergotamine	4.5
Adrenergic blocking agents, potentiators and antireleasers of catecholamines	Cocaine	10 —100
	Desipramine	30 —100
	Bretylium	10 —300
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The drugs used in this study were as follows. 1) *Adrenergic drugs*: dopamine hydrochloride, *l*-norepinephrine (base), *l*-epinephrine (base), *l*-isoproterenol hydrochloride, phenylephrine hydrochloride, tyramine hydrochloride, methamphetamine hydrochloride, ephedrine hydrochloride and methoxamine hydrochloride. 2) *Cholinergic drugs, and ganglion stimulating and blocking agents*: acetylcholine chloride, bethanechol chloride, carbachol chloride, methacholine chloride, 4-(*m*-chlorophenylcarbamoyloxy)-2-butylnyltrimethylammonium chloride (McN-A-343, McNeil Laboratories), nicotine (base), lobeline hydrochloride, tetramethylammonium chloride (TMA), 1,1-dimethyl-4-phenylpiperazinium iodide (DMPP) and tetraethylammonium bromide (TEA). 3) *Other principal biogenic amines and active polypeptides*: serotonin creatinine sulfate, histamine dihydrochloride, angiotensin II, lys-vasopressin (Sandoz), oxytocin (Syntocinon, Sandoz), bradykinin, kallikrein and eledoisin (Sandoz). 4) *Nucleosides and nucleotides*: ATP disodium, ADP trisodium, AMP disodium, adenosine (base), TPN (base), DPN (base), CTP trisodium, CDP sodium salts, CMP disodium, cytidine (base), UTP trisodium, UDP disodium, UMP disodium and uridine (base). 5) *Unspecific smooth muscle relaxants, analgesics and hypotensive drugs*: nitroglycerin (base), nicotinic acid, papaverine hydrochloride, verapamil hydrochloride, dipyrindamole (base), theophylline ethylenediamine, theobromine sodium salicylate, caffeine sodium benzoate, procaine hydrochloride, morphine hydrochloride and hydralazine hydrochloride. 6) *Adrenergic blocking agents, potentiators and antireleasers of catecholamines*: phentolamine methanesulfonate, phenoxybenzamine hydrochloride (SK & F), tolazoline hydrochloride, hydergine (Sandoz), ergotamine tartrate (Sandoz), cocaine hydrochloride, desipramine hydrochloride, bretylium tosylate and guanethidine sulfate. Ranges of the test doses of each compound were listed in Table 1. All doses were expressed as weights of base except for cytidine nucleotides and hydergine. One microgram of oxytocin corresponds to 0.46 IU⁵ and 1 μ g of lys-vasopressin to 0.27 IU.⁶ *l*-Norepinephrine was dissolved in 0.01 N HCl at a concentration of 1 mg/ml and diluted with 0.9% saline. All the other drugs except dipyrindamole and hydergine were dissolved in 0.9% saline. Injections of drug solutions were made into rubber tubing connected to the cannula (Fig. 1). A constant volume of 10 μ l was injected for 4 sec with 50 μ l microsyringes (Terumo, MSN-50). The 50 μ l-microsyringe is graduated in steps of 1 μ l. Since the solutions of oxytocin and lys-vasopressin contained chlorobutanol as preservative, control experiments were performed with chlorobutanol. The injection of chlorobutanol caused almost no response.

RESULTS

1) *Adrenergic drugs* (Fig. 2)

Dopamine, norepinephrine, epinephrine, phenylephrine, tyramine, methamphetamine, ephedrine and methoxamine caused a vasoconstriction, while isoproterenol produced a dilatation. Among them norepinephrine and epinephrine were almost equal in their potency for inducing a vasoconstriction. Small doses of norepinephrine and epinephrine dilated the femoral artery¹ but in the saphenous vascular bed a vasodilatation never occurred even in small doses of 0.001 to 0.003 μ g except in an experiment, in which small doses of epinephrine produced a weak dilatation. Dopamine and tyramine were approximately 1/100 in their vasoconstrictor activity as compared with that of norepinephrine and epinephrine.

2) *Cholinergic drugs, and ganglion stimulating and blocking agents* (Fig. 3)

One microgram of acetylcholine markedly dilated the saphenous vascular bed and similar effects were obtained with other choline esters, *i.e.*, bethanechol,

Fig. 2. Responses to adrenergic drugs of the saphenous vascular bed. P.P. stands for perfusion pressure.

Fig. 3. Responses of the saphenous vascular bed to cholinergic drugs, and ganglion stimulating and blocking agents.

carbachol and methacholine. Carbachol was the most potent among them. McN-A-343 also dilated the saphenous vascular bed but in only rather large doses. Among nicotinic ganglion stimulants, nicotine, lobeline and TMA caused a dilatation, while DMPP induced a vasoconstriction. TEA, a ganglion blocking agent, also caused a vasoconstriction.

3) Other principal biogenic amines and active polypeptides (Fig. 4)

Serotonin caused a constriction in a majority of experiments, but exceptionally a weak dilatation was observed. Histamine caused a marked vasodilatation. Angiotensin and vasopressin induced a slight and long-lasting constriction. Oxytocin had almost no effect even at $0.3 \mu\text{g}$. Bradykinin, eledoisin and kallikrein dilated the saphenous vascular bed.

Fig. 4. Responses of the saphenous vascular bed to other principal biogenic amines and active polypeptides.

Fig. 5. Responses of the saphenous vascular bed to nucleosides and nucleotides.

4) *Nucleosides and nucleotides* (Fig. 5)

Adenosine and adenine nucleotides caused a dilatation. Among them ATP was the most potent. Cytidine, CMP and CDP had almost no effect on the saphenous vascular bed. Only CTP caused a slight dilatation. TPN had no activity and DPN dilated the saphenous vascular bed. Uridine had no effect, and UMP, UDP and UTP usually caused a dilatation. Among them UTP was the most potent in vasodilator activity. However, 10 to 300 μg of UTP and 100 to 300 μg of UDP, UMP and uridine induced an apparent constriction in one experiment in which adenosine and adenine nucleotides induced a dilatation as usual.

5) *Unspecific smooth muscle relaxants, analgesics and hypotensive drugs* (Fig. 6)

Nitroglycerin elicited a significant dilatation. Nicotinic acid showed no activity even at a high dose of 300 μg . Papaverine produced a slight and transient dilatation.

Fig. 6. Responses of the saphenous vascular bed to unspecific smooth muscle relaxants, analgesics and hypotensive drugs.

Verapamil, a derivative of papaverine, and dipyridamole induced a slight and long-lasting dilatation. Among xanthine derivatives, theophylline, theobromine and caffeine dilated the saphenous vascular bed to a small degree. Procaine caused a slight dilatation and morphine caused a marked dilatation. Hydralazine, used clinically as a hypotensive drug, elicited at a dose of $100\text{ }\mu\text{g}$ a very gradual dilatation which reached the maximum in 7 to 8 min and continued for about 40 min. However, $30\text{ }\mu\text{g}$ of hydralazine showed no obvious effect.

Fig. 7. Responses of the saphenous vascular bed to adrenergic blocking agents, potentiators and antireleasers of catecholamines.

6) *Adrenergic blocking agents, potentiators and antireleasers of catecholamines* (Fig. 7)

In adrenergic blocking agents phenoxybenzamine caused no change and phentolamine caused a dilatation. It is worthy to note that tolazoline in doses of 1 to 10 μ g dilated the saphenous vasculature but at 30 μ g it produced a marked constriction following the dilatation. Ergotamine and hydergine induced a gradual and continuing constriction. Cocaine and desipramine constricted the saphenous vascular bed. The saphenous vasculature responded to guanethidine simply with a constriction, while it responded to bretylium differently in individual animals, that is, a dilatation occurred in three out of five experiments and a constriction in the other two experiments.

DISCUSSION

The saphenous artery is a main cutaneous branch of the femoral artery. Retrograde blood flow of the saphenous vascular bed measured about 1 ml/min when shunted to the femoral vein. Thus anastomoses with other arterial branches of the femoral artery are considered to be well developed. However, the pharmacological responses of the saphenous vascular bed were different from those of the femoral artery which supplies blood to both the skeletal muscles and the skin of the hindlimb. For example, in the saphenous vascular bed small doses of norepinephrine and epinephrine scarcely caused a vasodilatation, while the two agents in comparable dose ranges caused a dilatation in the femoral artery.¹ Therefore it is relevant that the responses of the saphenous vascular bed are characteristic of the cutaneous vessels but not of the muscle branches. Thus we consider that these

TABLE 2. *Classification of vasoactive compounds on the difference of*

Group a		Group b	Group c	
Coronary art.†	Dil.	Dil.	Dil.	
Renal art.†	Dil.	Const.	Const.	
Mesenteric art.†	Dil.	Dil.	Const.	
Femoral art.†	Dil.	Dil.	Dil.	
Saphenous art.	Dil.	Dil.	Const.	Dil.
ATP ADP		AMP	DMPP	Nicotine
UTP		Adenosine	Tolazoline	Lobeline
Bradykinin		DPN UDP		Hydralazine
Kallikrein		Dipyridamole		
Acetylcholine		Morphine*		
Histamine				
Isoproterenol				
Papaverine				
Nitroglycerin				
Theophylline				

* No effect on the coronary, mesenteric and femoral arteries.

† Quoted from Hashimoto and Kumakura.¹

anastomoses are mainly confined to those with other cutaneous branches of the femoral artery. The two naturally occurring catecholamines are known to have stimulant actions on both alpha- and beta-adrenergic receptors. The fact that the two catecholamines caused only a constriction of the saphenous vascular bed indicates that the alpha-adrenergic receptors predominate over the beta-adrenergic receptors in this vascular bed.

In Table 2 the pharmacological features of the saphenous vasculature are added to those of the coronary, renal, mesenteric and femoral arteries which were investigated by Hashimoto and Kumakura.¹ As evident from the table, the pharmacological characteristics of the saphenous vascular bed are distinct from those of the femoral artery in the following respect: The saphenous vasculature responded to DMPP, tolazoline and TEA with a vasoconstriction, while the femoral artery did with a vasodilatation.

DMPP and TEA are known to increase the release of norepinephrine from the sympathetic nerve endings.^{7,8} Tolazoline has some sympathomimetic properties producing tachycardia, an increase in cardiac output and a coronary dilatation, although the precise mechanism of the action has not been elucidated yet.⁹ In our study, the dilatation in relatively smaller doses of tolazoline may be ascribed to either sympatholytic or direct action but the constriction following the dilatation in moderate doses may be due to the release of norepinephrine. Celander and Folkow¹⁰ reported that a greater number of sympathetic constrictor fibers were distributed in the skin vessels as compared with the muscle vessels. Furthermore our results show that the alpha-adrenergic receptors are predominant in the

action in the coronary, renal, mesenteric, femoral and saphenous arteries

Group d		Group e	Group f	
Const.		Dil.	Const.	
Const.		Const.	Const.	
Dil.		Const.	Const.	
Dil.		Const.	Const.	
Const.	Dil.	Const.	Const.	Dil.
TEA	Procaine	Epinephrine	Angiotensin	UMP
		Norepinephrine	Vasopressin	
		Ephedrine	Methoxamine	
		Serotonin	Ergotamine	

Dil.: dilatation

Const.: constriction

saphenous vascular bed. Then it is reasonable that in our experiments compounds such as DMPP, tolazoline and TEA which probably increase the release of norepinephrine from the sympathetic nerve endings produce a distinct constriction.

Ganglion stimulating agents induced a constriction in the renal and mesenteric arteries, while in the saphenous vascular bed these compounds, other than DMPP, caused a dilatation. It is rather difficult to explain this difference, because it has been reported that, like DMPP, nicotine has an action stimulating the sympathetic nerves to cause the liberation of norepinephrine.¹¹ This difference in behavior between DMPP and the other ganglion stimulants must be elucidated in the future.

It is well known that both guanethidine and bretylium have a dual effect on the sympathetic nerve endings, *i.e.*, the initial release of and subsequent blockade of the release of catecholamines from the sympathetic nerve endings.¹² The initial sympathomimetic action of bretylium is less prominent and more transient than that of guanethidine.¹³ Besides, the direct muscarinic action of bretylium in large doses has already been reported.¹⁴ In accord with these, guanethidine caused a constriction of the saphenous vascular bed in our experiments, while bretylium produced a dilatation in most animals and occasionally a constriction.

Significant dilator responses were obtained with choline esters, adenine nucleotides and other vasodilator substances. But responses to uridine nucleotide were either a dilatation or a constriction depending upon the animals. It is worthy to note that morphine caused a marked dilatation characteristically in the saphenous vascular bed, although it caused a constriction in the renal artery and no effect in the coronary, mesenteric and femoral arteries (Table 2).

Acknowledgment

We wish to express our thanks to the Sandoz Yakuhin (Japan) for the supply of lys-vasopressin, elidoisin and ergotamine, to Dr. John Kleis of the McNeil Laboratories for McN-A-343, and to Mr. Gordon Wilfon of the Smith Klein & French Overseas Co. for phenoxybenzamine.

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