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ACTION OF SAUVAGINE ON THE MESENTERIC VASCULAR BED OF THE DOG

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SUMMARY

Sauvagine, a linear peptide of 40 amino acids, produced hypotension when administered intravenously to anesthetized dogs. Diastolic pressure was always more affected than systolic pressure. Aortic blood flow and venous return both increased to the same extent. The mechanism of the hypotensive response was mainly, if not exclusively, due to dilatation of the superior and inferior mesenteric arteries. Intravenous infusion of sauvagine in doses ranging from 3 to 10 ng · kg^{-1} · min^{-1} produced a dose-related increase of mesenteric blood flow up to 400% control values. Mucosal-submucosal blood flow of ileum and colon was increased, while blood flow in muscle was unaffected or slight decreased. The mesenteric vasodilator response was not prevented by adrenergic or muscarinic receptor blockade. The hypotensive response was more marked and sustained in dibenamine-propranolol treated dogs.

blood pressure; cardiac output distribution; mesenteric blood flow

INTRODUCTION

Sauvagine, a new amphibian peptide isolated from the skin of *Phyllomedusa sauvagei*, has the following amino acid sequence [1]:

Pyr–Gly–Pro–Pro–Ile–Ser–Ile–Asp–Leu–Ser–Leu–Glu–Leu–Leu–Arg–Lys–Met–Ile–Glu–Ile–Glu–Lys–Gln–Glu–Lys–Glu–Lys–Gln–Gln–Ala–Ala–Asn–Asn–Arg–Leu–Leu–Leu–Asp–Thr–Ile–NH_2

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In rats, sauvagine produces a long lastig hypotensive effect accompained by tachycardia and a reduction in urinary volume and renal excretion of electrolytes. The mechanism of the hypotensive action of sauvagine is not yet known, while tachycardia seems to depend, in part, upon sympathetic reflex, since propranolol decreases the heart rate in sauvagine-treated rats [2].

The aim of the present investigation was to study the cardiovascular effects of sauvagine in anesthetized dogs, with special reference to the mesenteric bed. Experiments were designed to determine whether the distribution of blood flow between the mucosal-submucosal and muscular layers of the intestinal wall is altered during sauvagine infusion.

MATERIAL AND METHODS

Twenty adult male mongrel dogs and 15 adult male beagle dogs, weighing between 9 and 32 kg were anesthetized with sodium pentobarbital (30 mg/kg i.v.) or sodium pentothal (25 mg/kg by i.v. injection followed by i.v. infusion of 25 $mg \cdot kg^{-1} \cdot h^{-1}$).

The animals were intubated and ventilated with a Palmer electronic respirator. A catheter was introduced into the left femoral vein for i.v. infusion of drugs.

Cardiovascular dynamics

Aortic pressure and mesenteric blood flow were measured in all dogs used. The 15 beagle dogs were divided in three groups of five dogs each. In the first group of animals ascending aortic flow, right and left ventricular pressure and left dp/dt were simultaneously recorded. In the second group inferior vena caval and carotid artery flow were recorded, whilst celiac and femoral artery flow were measured in the third group. In all dogs recording of cardiac activity, blood flow and blood pressure was performed before, during and after sauvagine infusion for periods not less than 60 min.

To record the mesenteric blood flow, the abdomen was opened through a midline incision and the superior and inferior mesenteric arteries exposed by careful dissection from surrounding tissue at the aortic origin. The ascending aorta, the inferior vena cava and the celiac artery were exposed by thoracotomy through the 5th left, 8th right and 10th left intercostal space, respectively.

Electromagnetic flow probes of appropriate sizes were placed around the vessels and connected to a Nicotron electromagnetic flowmeter. The zero reference point of blood flow was established periodically by brief mechanical occlusion of the vessels.

Aortic pressure, right and left ventricular pressure and left ventricular dp/dt were measured by means of catheters inserted through the carotid artery and cardiac walls. Catheters were connected to Statham pressure transducers (P23 Db) which were standardized for the level of the right atrium and calibrated against mercury. A Honeywell Accudata 132 Differentiator was used for differentiation of intraventricular blood pressure signals. When connected to a pressure amplifier, the differentiator provided the first derivative of blood pressure. All variables were recorded with an 18 channel Honeywell optical polygraph.

Mesenteric resistances were calculated dividing the mean aortic pressure by the mesenteric blood flow.

Cardiac output distribution

Distribution of cardiac output was measured by radioactive microspheres labeled with ^{141}Ce and ^{85}Sr according to the method of Greenway and Murty [3]. When two samples of microspheres labeled with two different radionuclides are injected into the left ventricle of an animal at different times, the distribution of the two radionuclides represents the distribution of cardiac output at the time of each injection. The radioactivity of two different isotopes can be measured separately using gamma-spectrometry. Thus this technique can be used to measure the relative distribution of cardiac output on two different occasions. In 20 mongrel dogs a bolus of radioactive microspheres (15 ± 2 μm diameter) in 20% dextran (3M-Nuclear Products, St. Paul, Minn.), counting about 5×10^6 cpm, was rapidly injected through a catheter inserted into the left ventricle. After each injection, the catheter was immediately washed with saline by a valve-operated automatic plastic syringe. A bolus of ^{141}Ce-labeled microspheres was injected between 2 and 5 min before sauvagine infusion in order to measure the distribution of cardiac output in basal condition. A second bolus of microspheres labeled with ^{85}Sr was injected upon maximum vasodilator response of the mesenteric vessels to sauvagine infusion.

Dogs were killed with pentothal and fragments of brain, tongue, lung, heart, liver, gallbladder, stomach, pancreas, spleen, kidney, muscle and skin were taken and dried in an oven at 180°C. The intestine was opened along the mesenteric border and cut into 50-cm segments. The muscle layer of each segment was stripped from the mucosal and submucosal layers, and samples of the layers were weighed and dried separately. About 0.5 g of dried tissue powder was counted with a dual channel gamma-spectrometer (Beckman Biogamma II).

Separation of the two isotopes in each sample was performed as follows. Since the main energy peak of gamma spectrum of ^{141}Ce is 145 keV and

^{85}Sr 514 keV, windows of the pulse-height analyzer were set at 5–350 keV (window A) and 350–600 keV (window B). At this setting ^{141}Ce was counted in window A whereas ^{85}Sr was counted in both windows A and B. At each time of counting, a standard of ^{85}Sr was counted in each window and the ratio of the count in each window (r) was calculated. The radioactivity of the two isotopes in each sample was calculated as:

$$^{141}Ce = C_A - \frac{C_B}{r}$$

$$^{85}Sr = C_B \left(1 + \frac{1}{r}\right) \quad (1)$$

where ^{141}Ce = cpm of ^{141}Ce in the tissue sample, ^{85}Sr = cpm of ^{85}Sr in the tissue sample, C_A = net counts collected in channel A (window A), C_B = net counts collected in channel B (window B), and r = ratio number of higher energy isotope (^{85}Sr) (i.e. C_B/C_A when ^{85}Sr is counted alone). Results were expressed as net cpm of each isotope per g dry tissue, and the ratio of cpm/g tissue to injected cpm was calculated for each isotope (R_{Sr} for ^{85}Sr and R_{Ce} for ^{141}Ce). The percent change of distribution of cardiac output per g tissue ($\% C_A$) during sauvagine infusion was then given by the equation:

$$\% C_A = (R_{Sr} - R_{Ce})/R_{Ce} \cdot 100 \quad (2)$$

Statistical analysis

Analysis of data was carried out using two statistical techniques. Differences in the mean values between treated and control groups in Tables I, II, III and VI were analyzed by the paired t-test. The data recorded in Table V were analyzed by the one-way analysis of variance.

Drugs

Pure, natural sauvagine, obtained from Farmitalia–Carlo Erba S.p.A. Research Lab., was dissolved in 0.9% saline solution and i.v. infused at a rate of 3, 5, 10, 50, 150, 300 ng $\cdot$ kg^{-1} $\cdot$ min^{-1} for 60 min. Norepinephrine-HCl (BDH, 0.02 mg/kg), propranolol-HCl (I.C.I., 2 mg/kg), dibenamine-HCl (*N*-(2-chloroethyl)dibenzylamine, BDH, 4 mg/kg) and atropine sulfate (BDH, 0.5 mg/kg) were injected i.v. in five dogs at different times before sauvagine infusion.

TABLE I

Aortic pressure response to i.v. infusion of sauvagine

Dose	Mean percent change ± S.E.M.						
($ng \cdot kg^{-1} \cdot min^{-1}$):	0	3	5	10	50	150	300
DP (mm Hg)	100 (93 ± 4)	−4.0 ± 1.0	−9.5 ± 5.5	−20.2 ± 4.2 *	−28 ± 4.2 **	−38.0 ± 6.1 **	−38.1 ± 4.3 **
SP (mm Hg)	100 (148 ± 5)	−4.5 ± 1	−3.5 ± 1.7	−12.6 ± 2.3 *	−17.0 ± 2 **	−30.5 ± 2.5 **	−28.6 ± 3.9 **

DP, diastolic pressure; SP, systolic pressure.

* $P < 0.05$.

** $P < 0.01$.

RESULTS

Cardiovascular response to i.v. infusion of sauvagine

No significant change in systemic blood pressure was observed at dose levels of 3 and 5 ng · kg^{-1} · min^{-1}. Doses of 10, 50, 150 and 300 ng · kg^{-1} · min^{-1} produced a fall in aortic blood pressure, with diastolic pressure decreasing more than systolic pressure, the maximum hypotensive response being reached at a dose level of 150 ng · kg^{-1} · min^{-1} (Table I).

In the animals infused with 10 ng · kg^{-1} · min^{-1} of sauvagine blood pressure attained the lowest level within the first 30 min of infusion and remained at this level as the infusion was continued. It slowly recovered 30–40 min after the end of the infusion. In dogs infused with 50, 150 and 300 ng · kg^{-1} · min^{-1} of the peptide, the hypotensive response reached a maximum with 5–10 min and decreased slowly by 30–40% in the last 30 min of sauvagine infusion. At these doses blood pressure returned to control values 60–90 min after the end of infusion.

Heart rate was unaffected and changes in left ventricular dp/dt were inconstant and statistically not significant. Left ventricular end-diastolic pressure was unchanged, whilst left ventricular end-systolic pressure always fell with the reduction in afterload. Right ventricular end-systolic pressure increased, whilst right ventricular end-diastolic pressure remained unchanged. During infusion of 150 ng · kg^{-1} · min^{-1} sauvagine, aortic blood flow showed an increase of 34.3 ± 5.4% and inferior vena caval flow an increase of 26.0 ± 4.1% (Table II).

TABLE II

Cardiovascular response to i.v. infusion of 150 ng · kg^{-1} · min^{-1} of sauvagine

	Control values	Mean percent change ± S.E.M.	*P* value
Heart rate	140 ± 14	+7 ± 3	>0.05
LV dp/dt (mm Hg/s)	1783 ± 397	+9.67 ± 6.4	>0.05
RV end-stystolic pressure (mm Hg)	21.5 ± 3.7	+12.4 ± 1.7	<0.05
RV end-diastolic pressure (mm Hg)	−1.5 ± 0.6	−1.7 ± 0.7	>0.05
LV end-systolic pressure (mm Hg)	124.0 ± 3.2	−9.3 ± 1.8	<0.05
LV end-diastolic pressure (mm Hg)	2.7 ± 2.3	−1.3 ± 3	>0.05
Ascending aortic flow (ml/min per kg)	85 ± 18	+34.3 ± 5.4	<0.01
Inferior vena caval flow (ml/min per kg)	66.5 ± 7.2	+26.0 ± 4.1	<0.01

TABLE III

Mean percent changes ± S.E.M. of flow and resistance in the superior mesenteric artery after i.v. infusion of sauvagine

Dose	Mean percent changes ± S.E.M.					
($ng \cdot kg^{-1} \cdot min^{-1}$):	0	3	5	10	50	150
Flow (ml per min kg^{-1})	100 (10.9 ± 1.4)	+24 ± 14.2 *	+127 ± 12.5 **	+276 ± 23.4 **	+299 ± 7.0 **	+155 ± 16.9 **
Resistance ($P/\emptyset$)	100 (0.97 ± 0.14)	−17.5 ± 8.51 *	−56.8 ± 4.9 **	−75 ± 3.8 **	−85 ± 3.5 **	−63 ± 2.6 **

P, systemic pressure; $\emptyset$, mesenteric artery flow.

* $P < 0.05$.

** $P < 0.01$.

Effects of sauvagine on regional blood flow

The marked vasodilatory effect of sauvagine on the superior mesenteric artery of the dog is shown in Table III. This effect was dose-related, showing a threshold at a dose of 3 ng · kg^{-1} · min^{-1} and reaching a maximum at a dose level of 50 ng · kg^{-1} · min^{-1}.

At all doses tested the vasodilatory response reached a plateau within 15–30 min and lasted as long as the infusion was continued (up to 60 min). Mesenteric blood flow and resistance recovered between 50 and 80 min after the end of the infusion.

The lower increase in mesenteric blood flow observed at doses of 150 ng · kg^{-1} · min^{-1} sauvagine compared with those of 50 ng · kg^{-1} · min^{-1} was obviously due to the decrease in aortic pressure which occurred at this dose level.

Mesenteric resistance showed a reduced response to the maximal dose tested, i.e. 150 ng · kg^{-1} · min^{-1} sauvagine, presumably on account of reflex sympathetic vasoconstriction.

TABLE IV

Percent change of distribution of cardiac output per g tissue after i.v. infusion of sauvagine (150 ng · kg^{-1} · min^{-1})

Vascular beds	% change
Right ventricle	+171.5 ± 13 **
Left ventricle	+66 ± 19 *
Septum	+58 ± 23
Liver	−48 ± 12 *
Stomach (fundus)	+24 ± 8
Stomach (antrum)	+35 ± 22
Duodenum	+130 ± 17 **
Ileum	+185 ± 25 **
Caecum	+170 ± 18 **
Colon	+542 ± 48 **
Rectum	+227 ± 42 **
Pancreas (head)	+95 ± 22 **
Pancreas (tail)	−24 ± 19
Spleen	−12 ± 9
Kidney	−2 ± 10
Brain	+41 ± 8 *
Skeletal muscle (legs)	−35 ± 11 *

* $P < 0.01$.
** $P < 0.05$.

The pattern of response of the inferior mesenteric artery to these doses of sauvagine was very similar to that of the superior mesenteric bed, though of lower magnitude. Increases of 27, 45, 82% of blood flow were observed in response to 5, 10, 50 ng · kg^{-1} · min^{-1} sauvagine, respectively. Celiac, hepatic and femoral artery blood flow decreased 20–60% during infusion of 50 ng · kg^{-1} · min^{-1} sauvagine, while renal and carotid flow remained unchanged.

The distribution of cardiac output measured by radioactive microspheres in different vascular beds during sauvagine infusion at a rate of 50 ng · kg^{-1} · min^{-1} is shown in Table IV. During sauvagine infusion blood was mainly diverted from liver and skeletal muscle to the mesenteric bed. The increase in coronary blood flow in response to sauvagine appeared to be more marked in the right than in the left ventricle, but this effect may be, in part, due to a reflex coronary response to the increased venous return and reduced left afterload. Cerebral blood flow showed a slight increase.

TABLE V

Percent distribution of cardiac output (mean ± S.E.M.) per g tissue

Sauvagine		Percent per g tissue				*P*
(ng/kg per min):		0	3	10	50	
Duodenum	Mc	0.21 ± 0.03	0.36 ± 0.02	0.78 ± 0.19	1.10 ± 0.27	<0.05
Duodenum	Ms	0.10 ± 0.05	0.03 ± 0.02	0.03 ± 0.01	0.27 ± 0.17	
Ileum 1	Mc	0.22 ± 0.06	0.33 ± 0.02	0.69 ± 0.18	0.94 ± 0.19	<0.05
Ileum 1	Ms	0.09 ± 0.04	0.03 ± 0.03	0.04 ± 0.01	0.22 ± 0.02	
Ileum 2	Mc	0.20 ± 0.05	0.33 ± 0.02	0.80 ± 0.12	0.84 ± 0.11	<0.01
Ileum 2	Ms	0.11 ± 0.07	0.01 ± 0.01	0.02 ± 0.01	0.24 ± 0.13	
Ileum 3	Mc	0.14 ± 0.02	0.24 ± 0.04	0.75 ± 0.08	1.05 ± 0.23	<0.01
Ileum 3	Ms	0.17 ± 0.11	0.11 ± 0.08	0.11 ± 0.08	0.23 ± 0.17	
Ileum 4	Mc	0.14 ± 0.04	0.21 ± 0.01	0.74 ± 0.09	0.70 ± 0.05	<0.01
Ileum 4	Ms	0.17 ± 0.09	0.11 ± 0.08	0.03 ± 0.01	0.20 ± 0.13	
Caecum		0.10 ± 0.03	0.21 ± 0.02	0.39 ± 0.07	0.63 ± 0.05	<0.01
Colon 1	Mc	0.11 ± 0.05	0.38 ± 0.01	0.83 ± 0.09	2.01 ± 0.17	<0.01
Colon 1	Ms	0.12 ± 0.04	0.13 ± 0.02	0.19 ± 0.01	0.18 ± 0.10	
Colon 2	Mc	0.20 ± 0.09	0.41 ± 0.08	0.89 ± 0.10	1.97 ± 0.13	<0.01
Colon 2	Ms	0.05 ± 0.02	0.04 ± 0.01	0.05 ± 0.01	0.01 ± 0.01	
Rectum	Mc	0.19 ± 0.06	0.29 ± 0.04	0.63 ± 0.27	0.94 ± 0.13	<0.05
Rectum	Ms	0.04 ± 0.01	0.03 ± 0.02	0.04 ± 0.02	0.03 ± 0.01	

Mc, mucosal layer; Ms, muscle layer.

TABLE VI

Effects of adrenergic blockade on cardiovascular responses to i.v. infusion of sauvagine (dibenamine, 4 mg/kg i.v., propranolol, 1 mg/kg i.v.)

	Controls	Dibenamine + propranolol	Sauvagine (ng/kg per min)		Sauvagine (ng/kg per min) + dibenamine + propranolol	
			10	150	10	150
Mesenteric flow (ml/kg per min)	10 ± 3	12 ± 6	29 ± 6	21 ± 5	26 ± 7	22 ± 4
Mesenteric resistance ($R = P/F$)	1.1 ± 0.3	0.9 ± 0.2	0.3 ± 0.1	0.4 ± 0.1	0.3 ± 0.1	0.3 ± 0.1
Diastolic pressure (mm Hg)	87 ± 3	69 ± 7	70 ± 6	54 ± 3	45 ± 4	38 ± 3
Systolic pressure (mm Hg)	144 ± 8	132 ± 14	130 ± 7	103 ± 9	121 ± 11	95 ± 4

The effect of sauvagine on the distribution of blood flow between the muscle layer and submucosal-mucosal layer of the various intestinal tracts is illustrated in Table V. Data presented indicate that sauvagine produced selective mucosal-submucosal vasodilatation but did not affect muscle vessels. The vasodilatory response was partially dose-dependent, and for each dose, the colon was the area most affected.

At the dose level of 50 ng · kg^{-1} · min^{-1} the percent distribution of cardiac output to colon mucosa increased about 10-fold.

Effects of adrenergic and muscarinic blockade on cardiovascular response to sauvagine

The hypotensive and mesenteric vasodilator response to i.v. infusion of sauvagine (10 and 150 ng · kg^{-1} · min^{-1}) were studied before and after propranolol (1 mg/kg), dibenamine (4 mg/kg) and atropine (1 mg/kg) in 5 beagle dogs.

When sympathetic block was established (1 h after dibenamine and propranolol injections; Table VI), the mesenteric blood flow increased only about 10–20% during the control period. The contribution of the sympathetic vasoconstriction to the resting tone of the intestinal resistance vessels in the anesthetized dog is probably relatively small.

It was not surprising, therefore, that propranolol and dibenamine, either alone or in combination, had little effect upon the intensity or the duration of the mesenteric vasodilator response to sauvagine.

Dibenamine plus propranolol, however, produced a more marked and sustained hypotensive response to the peptide, presumably through removal of reflex vasoconstriction in peripheral vascular beds. Atropine did not modify either the mesenteric vasodilation or the hypotensive response to sauvagine.

DISCUSSION

Sauvagine, a new amphibian straight chain peptide of 40 amino acids, does not appear to display any similarity to other vasoactive peptides from amphibian skin and mammals.

The present data indicate that sauvagine given i.v. to anesthetized dogs produces a fall in systemic arterial blood pressure with no significant effects on the heart. Diastolic pressure was always more affected than systolic pressure whereas aortic blood flow and venous return both increased to the same extent.

These observations point to dilatation of the arteriolar vessels as the

cause of the hypotensive response and the increase in venous return. Direct measurements of the potent dilatatory effect of sauvagine on the mesenteric bed confirmed this view. In most sauvagine-treated dogs diastolic pressure decreased when mesenteric blood flow increased 70–100%. Regional vasodilation of this magnitude seems to be necessary for a systemic hypotensive response on account of a reflex increase in sympathetic tone. Increased sympathetic tone may also explain why the mesenteric vasodilator response lasted longer than the hypotensive response. The role of sympathetic vasoconstriction in the pressure response was confirmed in dibenamine-treated dogs, in which sauvagine infusion produced a more prompt and marked drop in systemic blood pressure which lasted as long as mesenteric vasodilation persisted. The dilatation produced by the peptide in the mesenteric arterial bed appeared to be confined to the mucosal-submucosal layer of the ileum and colon and insensitive to muscarinic and adrenergic receptor blockade.

The latter observation indicates that sauvagine induced vasodilation is not caused by release of acetylcholine or catecholamines, although involvement of other mediators cannot be excluded.

The action of sauvagine on the cardiovascular system of the dog is similar to that described by MacCannell and Lederis for urotensin I, a peptide derived from the urophysis of bony fish [4].

Although the amino acid sequence of this peptide is unfortunately not yet known, the amino acid content appears to differ significantly from that of sauvagine because of the presence of aromatic amino acid residues. An antibody to sauvagine was recently produced in rabbits which cross-reacted with the purified extract of urophysis. Preliminary immunohistochemical data obtained in the *Tinca tinca* urophysis, using this antibody, indicate the presence of sauvagine-like immunoreactivity in the large neurons of this organ [5].

There seem to be species differences in the cardiovascular response to sauvagine since in the pentothal-anesthetized dog the hypotensive response was less intense and shorter than in the urethane-anesthetized rat. In the rat sauvagine induces a less selective peripheral vasodilation, i.e. femoral rather than mesenteric, as well as intense tachycardia.

To our knowledge, the effects of sauvagine on man are not yet known, and the species differences between rat and dog discourage extrapolation of basic pharmacologic knowledge of the effects of sauvagine in animal models to normal and (or) diseased man. Nevertheless, the peptide may represent a useful pharmacological tool in investigations on gastrointestinal physiology, at least in the dog. An agent producing a dose-related increase in gut blood flow might contribute to a better understanding of correlations between mesenteric blood flow and intestinal absorption.

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