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Selective vasoconstriction by alniditan in the carotid vascular bed of anaesthetized dogs

André Van de Water, Jan D'Aubioul, Willy Van Gerven, Karel Van Ammel, Fred De Clerck *

Department of Cardiovascular and Pulmonary Pharmacology, Janssen Research Foundation, Turnhoutseweg 30, B-2340 Beerse, Belgium

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Abstract

In anaesthetized dogs, alniditan or (−)-(*R*)-*N*-[3,4-dihydro-2*H*-1-benzopyran-2-yl)methyl]-*N'*-(1,4,5,6-tetrahydro-2-pyrimidinyl)-1,3-propanediamine dihydrochloride, a new compound with 5-HT_1-like receptor ligand effects, dose dependently (0.63–80 μg/kg i.v.) reduced common carotid arterial blood flow with comparatively little effect on other cardiovascular variables including coronary, mesenteric and renal arterial blood flow, systemic and pulmonary vascular resistance and airway resistance. The potency of alniditan was higher than that of sumatriptan and comparable to that of ergotamine (dose producing a 50% reduction: alniditan = 5.1 μg/kg i.v.; sumatriptan = 13.1 μg/kg i.v.; ergotamine = 4.6 μg/kg i.v.; median values). The reduction of carotid arterial blood flow by alniditan was accompanied by an increase of carotid arterial vascular resistance and correlated with the increase of the difference in oxygen saturation between arterial and jugular venous blood, suggesting a preferential reduction of extracerebral shunt flow by the compound via constriction of arteriovenous anastomoses in the carotid vascular region. The extent and duration of carotid arterial blood flow reductions after alniditan at 5 μg/kg i.v. were similar to those after sumatriptan 15 μg/kg i.v. but larger/longer after alniditan at 15 μg/kg i.v. than after sumatriptan at 15 μg/kg i.v. The dose-dependent increase of carotid arterial vascular resistance by alniditan was similar in dogs premedicated daily for 4 days with solvent or active compound (20 μg/kg i.v.), indicating absence of tolerance or resetting of sensitivity to the compound.

Keywords: Alniditan; Sumatriptan; Ergotamine; Carotid arterial resistance

1. Introduction

At low concentrations in vitro (ED_{50}-values between 6–10 nM), the new non-indole chemical compound alniditan or (−)-(*R*)-*N*-[3,4-dihydro-2*H*-1-benzopyran-2-yl)methyl]-*N'*-(1,4,5,6-tetrahydro-2-pyrimidinyl)-1,3-propanediamine dihydrochloride (R091274; Janssen Research Foundation, Belgium; Fig. 1) induces contractions of saphenous veins, basilar and middle cerebral arteries of canine and porcine origin with lesser or no effects on the tonus of various other blood vessels and tissues (W. Janssens and F. De Clerck, personal communication).

In radioligand experiments, alniditan displays a high affinity for 5-HT_{1D} receptor sites from calf brain (K_i = 1.1 nM), a lower affinity for cloned human 5-HT_{1A} receptor sites (K_i = 4.1 nM) and a poor affinity for a variety of other receptor binding sites (J. Leysen, personal communication). These observations suggest that the effects of alniditan on isolated vascular tissues might involve an effect on 5-HT_1-like receptors.

In the present study, we therefore analyzed the overall cardiovascular and pulmonary effects of alniditan administered intravenously to anaesthetized dogs with particular focus on changes in flow and resistance in the carotid and coronary arterial vascular bed. We compared the effects of this novel molecule with those of sumatriptan, a comparatively selective agonist at 5-HT_{1D}-like receptors (Humphrey et al., 1989; Saxena and Den Boer, 1991; Feniuk et al., 1989, 1991) and of ergotamine, a mixed agonist/antagonist at various receptor subtypes (Saxena and Den Boer, 1991; Feniuk et al., 1991). The latter two compounds are used clinically for the abortive treatment of migraine attacks (Saxena and Den Boer, 1991; Feniuk et al., 1991).

* Corresponding author. Tel.: 014/60 26 52; fax: 014/60 28 41.

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2. Materials and methods

2.1. Haemodynamic studies

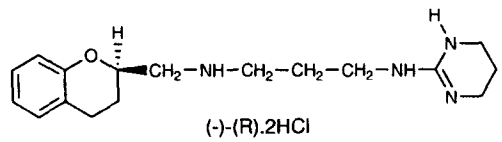
2.1.1. Animal preparation

The experiments were performed on adult Beagle dogs of either sex and varying age, ranging in body weight from 11 to 18 kg (median 12.8). The animals were anaesthetized with a mixture of 0.015 mg/kg scopolamine and 0.05 mg/kg lofentanil and relaxed with succinylcholine (1 mg/kg), all given intravenously. Central body temperature was monitored with a thermistor positioned in the pulmonary artery and was kept at 36°C with a heated water mattress. The animals were intubated with a cuffed endotracheal tube. Intermittent positive pressure ventilation was performed with a mixture of pressurised air and oxygen (60/40) using a volume-controlled ventilator (Siemens-Elema, Sweden). The CO_2 concentration in the expired air as determined with a capnograph (Siemens-Elema CO_2 analyser 930) was kept at 5 vol% by adjustment of the respiratory volume (respiratory rate = 20 breaths/min). The maximal pressure (cm H_2O) required for such a volume-adjusted ventilation was used to calculate compound-induced changes in airway pressure indicative for changes in airway resistance. Immediately after the induction of anaesthesia, a continuous intravenous infusion of 5 mg/kg/h of etomidate was initiated and small additional doses of fentanyl (0.02 mg/kg i.v.) were given at 90-min intervals. Heparin (500 IU/kg i.v.) was administered to prevent blood coagulation while sodium bicarbonate was administered intravenously as necessary to correct for base deficits. Gentran 40 with glucose 5% was continuously infused intravenously at a rate of 100 ml/h to compensate for fluid loss.

2.1.2. General haemodynamic analysis

In a first series of experiments, the effects of alniditan, sumatriptan, ergotamine and their solvent on the general haemodynamic status and on carotid and coronary arterial blood flow as well as vascular resistance were analyzed.

After thoracotomy through the 4th left intercostal space, the heart was suspended in a pericardial cradle and the left circumflex coronary artery was dissected free about 2 cm distal to its offspring. Pre-calibrated, well-fitting electromagnetic flow probes (Janssen Scientific Instruments) were placed around the left circumflex coronary artery and left common carotid artery. Blood flow in the common carotid (in ml/min) and in the left circumflex coronary artery (in ml/min) was measured by means of square-wave electromagnetic flow meters (Janssen Scientific Instruments). Mean blood flow was determined by the computerized integration of the instanteneous flow curve. Zero flow baseline was established by a brief, total occlusion of the common carotid artery and left circumflex coronary artery distal to the flow probe.



(-)-(R).2HCl

Alniditan

Fig. 1. Structure and chemical name of alniditan (R091274, Janssen Research Foundation, Belgium).

The electrocardiogram was derived from limb leads (standard lead II) and was used for the computerised calculation of heart rate (in beats/min), and of the duration of the PQ-, QRS-, QT- and QTc-intervals (in ms). Maximal changes in the ST-segment (in mV) and in the amplitude of the T-wave (in ms) of the ECG after medication relative to the premedication value were measured manually from the chart recordings.

Left ventricular and ascending aortic blood pressures were measured by retrograde catheterisation of the appropriate region via the right carotid and the left femoral artery, respectively, with high fidelity catheter-tip micromanometers (Drager-Ballings, Netherlands). Zero-line calibration was achieved by simultaneously recording the pressure signal through the lumen of the catheter with an external pressure transducer (Gould P23ID, Oxnard, USA) positioned at the mid-chest level and equating both zero lines on the recorder. Through a femoral vein, a Swan-Ganz balloon-guided thermistor catheter (Santa Ana, USA) was placed in the pulmonary artery to measure pulmonary artery blood pressure via an external pressure transducer (Gould P23ID) positioned at the mid-chest level and cardiac output by means of the ECG- and respiration-triggered thermodilution technique (Janssen Scientific Instruments, Belgium). The other femoral vein was cannulated for the injection into the right atrium of saline at room temperature for thermodilution measurements and of the compounds under scrutiny or their solvent. The right jugular vein and the femoral artery were cannulated for blood sampling. All catheters were placed in position under radioscopic control. Continuous registration of the analogue signals was performed on an eight-channel ink-jet recorder (Mingograph 800, Siemens-Elema). Via transducer amplifiers, blood pressure, temperature, blood flow and ECG signals were fed into a digital minicomputer (PDP 11/23, DEC, USA), connected to a dual diskette drive and a MacIntosh II computer (Apple, USA). The following variables were calculated on line and printed on a matrix printer (Facit 4512B, Belgium), usually at 1 min intervals: heart rate (in beats/min), systolic, diastolic and mean aortic blood pressure (in kPa), systolic and diastolic pulmonary artery blood pressure, left ventricular end-diastolic pressure (in kPa), the maximum positive and maximum negative rate of change of isovolumic left ventricular pressure (in kPa/s), the maximum first derivative divided by the actually developed pressure in the left ventricle (in /s), pressure rate product (in kPa × beats/min), cardiac output (in l/min) and stroke volume (in ml). The time

constant of relaxation (in ms) was calculated as previously described (Thompson et al., 1982). The systemic vascular resistance (in kPa/l × min) and pulmonary vascular resistance (in kPa/l × min) were calculated with the use of standard formulae. Vascular resistance to flow (kPa/ml × min) in the carotid and coronary vasculature was calculated by dividing mean arterial pressure by the mean arterial flow in each vessel. All values were simultaneously transmitted to a diskette and were plotted after the experiments on a digital *x–y* plotter (HP 7475A, Hewlett-Packard, USA) (Van de Water et al., 1992).

Before and 15 min after medication, blood was sampled simultaneously from the right jugular vein and from the femoral artery. Blood gas analysis was performed using an ABL 300 Radiometer analyzer. From these data, the arteriovenous difference in oxygen saturation (%) after the various medications was calculated as a parameter for changes in flow via arteriovenous anastomoses in the carotid vascular bed (Saxena and Verdouw, 1982; Den Boer et al., 1991). After instrumentation, the preparations were allowed to stabilize for 20 min with continuous registration of the premedication baseline values. Thereafter, the animals were allocated to one of the following medication groups: (1) solvent ($n = 9$); (2) alniditan ($n = 9$); (3) sumatriptan ($n = 9$); (4) ergotamine ($n = 7$). In each group, increasing doses of solvent (4, 16, 63, 250 μl/kg), alniditan, sumatriptan or ergotamine (1.25, 5, 20, 80 μg/kg) were administered by intravenous bolus injection at 30–45 min intervals.

2.1.3. Regional haemodynamic studies

In a second set of experiments, the effects of alniditan, sumatriptan and their solvent on blood flow through the carotid, mesenteric and renal arteries were analyzed. For that purpose, blood flow was measured in the left common carotid and superior mesenteric and left renal arteries using Doppler flow probes in closed-chest dogs anaesthetized as described above. After a stabilisation period, the animals received one of the following medications: (1) solvent ($n = 7$); (2) alniditan ($n = 8$); (3) sumatriptan ($n = 10$). In each group, increasing doses of solvent (4, 16, 63, 250 μl/kg), alniditan or sumatriptan (1.25, 5, 20, 80 μg/kg) were administered by intravenous bolus injection at 45 min intervals.

2.1.4. Duration of action

In a third set of experiments, the duration of action of alniditan and of sumatriptan in reducing blood flow in the carotid vascular bed was analyzed. For that purpose, blood flow in the left common carotid artery, heart rate, systolic and diastolic aortic pressure and ECG characteristics were measured in closed-chest, anaesthetized dogs. After a stabilisation period, the animals were allocated to medication with (1) solvent 63 μl/kg i.v. ($n = 5$); (2) alniditan 5 μg/kg i.v. ($n = 5$); (3) alniditan 15 μg/kg i.v. ($n = 5$); (4) sumatriptan 15 μg/kg i.v. ($n = 5$). In each group, values of the various parameters and variables were recorded before and up to 240 min after medication.

2.1.5. Repeated medication

In a last paradigm, the potential effect of repeated medication with alniditan on the acute increase in carotid vascular resistance elicited by that compound was analyzed. For that purpose, awake dogs were medicated daily for 4 days via the intravenous route with solvent (63 μl/kg; $n = 12$) or alniditan (20 μg/kg; $n = 8$). On the fifth day, approximately 24 h after the last medication, the animals were anaesthetized and instrumented for the measurement of carotid arterial blood flow and of overall haemodynamic parameters. After a stabilisation period, animals pretreated with solvent received increasing doses of solvent (2, 4, 16, 63, 250 μl/kg i.v.; $n = 6$) or alniditan (0.63, 1.25, 5, 20, 80 μg/kg i.v.; $n = 6$) at 45 min intervals. Those premedicated with alniditan 20 μg/kg i.v. daily for 4 days received increasing doses of alniditan (0.63, 1.25, 5, 20, 80 μg/kg i.v.; $n = 8$). Changes in common carotid blood flow and resistance as well as in coronary arterial and systemic vascular resistance were determined using the premedication values as control values.

2.2. Data analysis

Results are expressed as means ± S.E.M. or as medians obtained in the stated number (n) of experiments.

Premedication baseline values of each group in the various experimental set-ups were evaluated for statistical differences using the Mann-Whitney U-test on differences. The values after the administration of the various doses of compounds or their solvent were analyzed for an overall statistical significant difference versus the premedication values using Friedman's two-way analysis of variance by ranks. In case such a difference was present, the changes after medication relative to the premedication values in the compound groups were compared with those in the solvent group using the Mann-Whitney U-test on differences. Bonferroni's inequality was applied to the multiple comparisons procedure, P-values equal to or smaller than 0.05/4 comparisons = 0.0125 being considered to reflect statistical significance.

In each medication group, the peak changes elicited by solvent or compounds in the values of the various parameters including carotid and coronary arterial blood flow and resistance, systemic and pulmonary vascular resistance, mesenteric and renal blood flow and difference in oxygen saturation between arterial and jugular venous blood were calculated relative to the appropiate premedication baseline values. These pre/postmedication changes induced by solvent, alniditan, sumatriptan or ergotamine were evaluated for statistical significance using the Mann-Whitney U-test for intergroup comparisons. Data not normally distributed throughout the population under study (ST-segment and

T-wave amplitude of the ECG) were analysed using Kruskal and Wallis one way analysis of variance. *P*-values equal to or smaller than 0.05 were considered to reflect statistically significant differences.

In order to compare potencies of the various compounds, peak responses of carotid arterial blood flow to various doses of compound in each medication group were used to compute the median dose producing a reduction of 50% (ED_{50}) by means of probit analysis. Correlations between changes in blood flow/resistance and oxygen saturation differences were calculated according to Pearson (Milliken and Johnson, 1984; Siegel, 1956).

2.3. Compounds

Alniditan ((−)-(*R*)-*N*-[3,4-dihydro-2*H*-1-benzopyran-2-yl)methyl]-*N'*-(1,4,5,6-tetrahydro-2-pyrimidinyl)-1,3-propanediamine dihydrochloride, Janssen Research Foundation, Belgium; Fig. 1), sumatriptan (Glaxo, UK) and ergotamine (Sigma, USA) were dissolved in saline (NaCl 0.9%) at a concentration of 0.32 mg/ml. Saline was used in the solvent-treated control groups.

3. Results

3.1. General haemodynamic effects

In comparison with the baseline values, the intravenous administration of solvent to anaesthetized dogs caused no relevant changes in haemodynamic variables throughout the experimental observation period (data not shown). In comparison with the intravenous administration to anaesthetized dogs of solvent ($n = 9$; Fig. 2A,B,C), that of alniditan ($n = 9$; Table 1; Fig. 2A) or sumatriptan ($n = 9$; Table 2; Fig. 2B) in doses of 1.25–80 μg/kg i.v. had little or no consistent dose-related effect on heart rate, systolic and diastolic aortic pressure, cardiac contractile performance and relaxation, left ventricular end-diastolic pressure, stroke volume, cardiac output, pressure-rate product, systolic and diastolic pulmonary arterial pressure, systemic and pulmonary vascular resistance to flow, coronary blood flow or coronary vascular resistance to flow, arterial oxygen pressure and airway resistance.

By contrast, relative to solvent both alniditan and sumatriptan elicited a prominent and dose-related reduction of blood flow in the common carotid artery (Tables 1 and 2) and an increase of resistance in that vascular bed from 1.25 μg/kg i.v. on (Fig. 2A,B). The peak reductions of common carotid artery blood flow and the concomitant increase of common carotid arterial resistance elicited by alniditan at 5 and 20 μg/kg i.v. were significantly more pronounced than those elicited by similar doses of sumatriptan. Also, the calculated dose of alniditan eliciting a 50% reduction of common carotid artery blood flow (ED_{50}: median 5.1 μg/kg i.v.) was significantly lower than that of sumatriptan (ED_{50}: median 13.1 μg/kg i.v.).

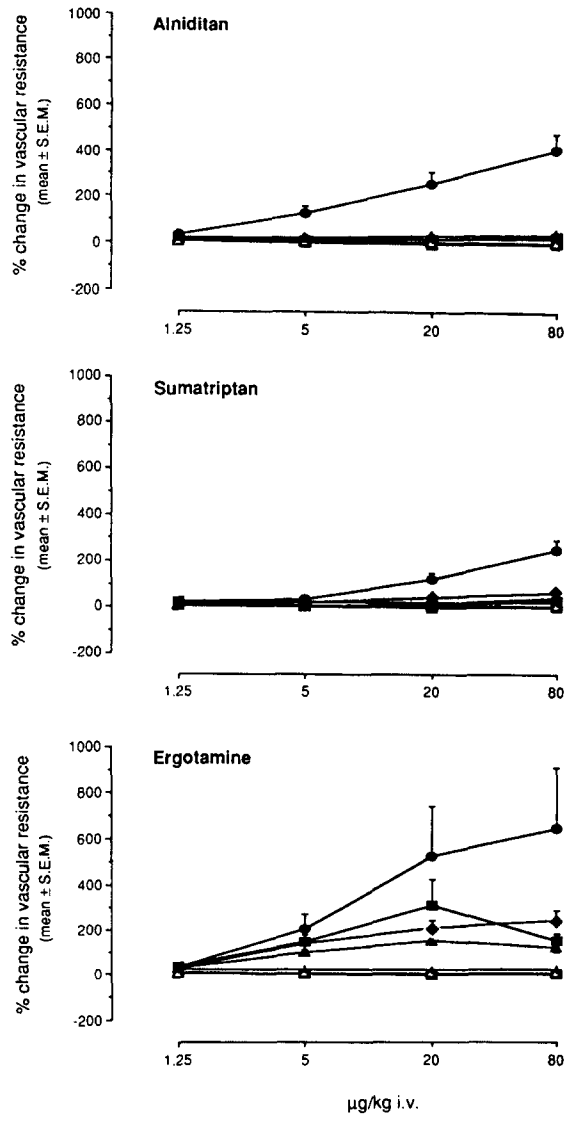


Fig. 2. Comparative effects of alniditan, sumatriptan and ergotamine on carotid arterial, coronary arterial, systemic and pulmonary vascular resistance in anaesthetized dogs. Percentages of change versus premedication values of common carotid (○;●) and coronary (□;■) arterial vascular resistances and systemic (△;▲) and pulmonary (◇;◆) vascular resistances induced by solvent (open symbols; $n = 9$), or by alniditan ($n = 9$), sumatriptan ($n = 9$) and ergotamine ($n = 7$) (full symbols). Means + S.E.M. Relative to solvent, alniditan and sumatriptan 1.25–80 μg/kg i.v. significantly increase common carotid arterial vascular resistance in a dose-related fashion without relevant effects on the other vascular beds. Resistance increases in the carotid vascular bed elicited by alniditan at 5 and 20 μg/kg i.v. are significantly more pronounced than by sumatriptan at these doses. Relative to solvent, ergotamine significantly increases carotid arterial and pulmonary vascular resistance as well as coronary arterial and systemic vascular resistance (5–80 μg/kg i.v.).

As compared to that of solvent, the intravenous administration of ergotamine (1.25–80 μg/kg i.v.) elicited changes not only in carotid arterial blood flow and resistance but also in coronary arterial blood flow and resistance as well as in general haemodynamic and pulmonary

variables (Table 3; Fig. 2C). Indeed, common carotid artery blood flow was significantly reduced (ED_{50}: median 4.6 μg/kg i.v.) and common carotid arterial resistance significantly increased from 1.25 and 5 μg/kg i.v. on, respectively. Ergotamine-induced changes in common carotid artery blood flow and common carotid arterial resistance were of a similar extent as those elicited by alniditan over the whole dose range examined but more pronounced than those induced by sumatriptan at 5 and 20 μg/kg i.v. However, in contrast to alniditan and sumatriptan, ergotamine also significantly increased systemic and pulmonary vascular resistance to flow, reduced coronary blood flow and augmented coronary vascular resistance to flow, increased airway pressure and reduced heart rate as well as cardiac contractile performance and relaxation from 5 μg/kg i.v. on (Table 3).

Table 1
Haemodynamic and pulmonary parameters before (B) and after (A) the intravenous administration of alniditan to anaesthetized dogs

Parameter	Alniditan (μg/kg i.v.)			
	1.25	5	20	80
HR (beats / min)				
B	104	116	105	106
A	99	109	106	113
AoPs (kPa)				
B	16.7	16.8	15.5	16.4
A	16.8	16.1	16.1	15.6 [a]
AoPd (kPa)				
B	9.9	9.9	8.7	9.5
A	9.7	9.7	8.5	8
PaPs (kPa)				
B	3.3	3.1	2.7	3.1
A	3.3	2.8	2.8	2.4
PaPd (kPa)				
B	1.1	1.2	1.3	1.1
A	1.1	1.1	1.2	1.1
LVEDP (kPa)				
B	1.5	1.5	1.6	1.5
A	1.5	1.5	1.7	1.5
LVdp / dtmax (kPa / s)				
B	429	429	367	412
A	417	385	367	376
LVdp / dtmax / pd (/ s)				
B	56	58	56	57
A	55	55	54	57
SV (ml)				
B	14	14	12	12
A	14	13	13	12
CO (l / min)				
B	1.6	1.8	1.4	1.3
A	1.6	1.5	1.4	1.5
PRP (× 1000) (in kPa × b / min)				
B	1.8	1.8	1.7	1.8
A	1.8	1.8	1.8	1.7
SVR (kPa / l × min)				
B	8.3	8.4	8.2	8.3
A	8.1	8.5	9.2	10.1
PVR (kPa / l × min)				
B	1.3	1.2	1.5	1.5
A	1.4	1.4	1.5	1.5
LVdp / dtmin (kPa / s)				
B	322	280	235	275
A	322	289	265	250
τ relaxation (ms)				
B	31	30	30	31
A	31	32	30	30
AP (cmH_2O)				
B	15.3	15.8	16.7	17.4
A	15.4	15.8	16.7	17.4
LCXBF (ml / min)				
B	30	32	32	27
A	30	30	27	26
CCBF (ml / min)				
B	116	98 [a]	57	39
A	99 [a]	63 [a]	41	28

Notes to Table 1:
Median values ($n = 9$) before (B) and at peak effect after (A) the intravenous administration of increasing doses of alniditan at 30–45 min intervals. Abbreviations: HR = heart rate; AoPs = systolic aortic blood pressure; AoPd = diastolic aortic blood pressure; PaPs = systolic pulmonary artery blood pressure; PaPd = diastolic pulmonary artery blood pressure; LVEDP = left ventricular-end diastolic pressure; LVdp/dtmax and LVdp/dtmin = maximum positive and maximum negative rate of change of isovolumic left ventricular pressure; LVdp/dtmax/pd = LVdp/dtmax divided by the actually developed pressure in the left ventricle; SV = stroke volume; CO = cardiac output; PRP = pressure rate product; SVR = systemic vascular resistance; PVR = pulmonary vascular resistance; τ = time constant of relaxation; AP = airway pressure; LCXBF = left circumflex coronary artery blood flow; CCBF = common carotid artery blood flow. [a] Significantly different from solvent.

In the dose range tested (1.25–80 μg/kg i.v.), alniditan, sumatriptan and ergotamine produced no consistent changes relative to solvent in the duration of the PQ-, QRS-, QT- or QTc-intervals of the ECG. The incidences of changes in the amplitude of the ST-segment and the T-wave of the ECG relative to premedication values did not differ significantly between solvent, alniditan, sumatriptan or ergotamine (results not shown).

3.2. Blood gas analysis

Relative to solvent, ergotamine (20 and 80 μg/kg i.v.) but not alniditan and sumatriptan (1.25–80 μg/kg i.v.) significantly reduced arterial oxygen saturation. Alniditan (1.25–80 μg/kg i.v.), sumatriptan (20 and 80 μg/kg i.v.) and ergotamine (5–80 μg/kg i.v.) significantly reduced the oxygen saturation in jugular venous blood. Consequently, alniditan (1.25–80 μg/kg i.v.), sumatriptan (20 and 80 μg/kg i.v.) as well as ergotamine (5–80 μg/kg i.v.) caused a dose-related increase in the difference in oxygen saturation between arterial and jugular venous blood. The latter change elicited by either alniditan, sumatriptan or ergotamine correlated significantly with the reduction of common carotid artery blood flow elicited by each compound (Fig. 3). Also, the increase of the arteriovenous oxygen saturation difference produced by each compound correlated significantly with the increase of the common carotid arterial resistance (Pearson correlation

Table 2
Haemodynamic and pulmonary parameters before (B) and after (A) the intravenous administration of sumatriptan to anaesthetized dogs

Parameter	Sumatriptan (μg/kg i.v.)			
	1.25	5	20	80
HR (beats / min)				
B	101	109	101	102
A	102	105	108	91
AoPs (kPa)				
B	19.1	17.1	17.2	16.7
A	17.5	17.1	16.4	16.6
AoPd (kPa)				
B	11.6	10	9.5	8.4
A	11.5	9.8	8.8	8.4
PaPs (kPa)				
B	3.2	3.4	3.4	3.2
A	3.1	3.3	3	3
PaPd (kPa)				
B	0.7	1	1	1
A	0.7	1	1	0.9
LVEDP (kPa)				
B	1.5	1.4	1.6	1.6
A	1.4	1.6	1.6	1.6
LVdp / dtmax (kPa / s)				
B	346	321	300	311
A	340	319	289	296
LVdp / dtmax / pd (/ s)				
B	48	53	53	56
A	49	52	54	56
SV (ml)				
B	12	12	12	12
A	12	13	12	11
CO (l / min)				
B	1.3	1.4	1.3	1.1
A	1.3	1.4	1.3	1
PRP (× 1000) (kPa × b / min)				
B	1.8	1.8	1.6	1.5
A	1.8	1.7	1.3	1.4
SVR (kPa / l × min)				
B	10.5	8.5 [a]	8.6	10
A	11	8.7	8.7	11.2
PVR (kPa / l × min)				
B	1.2	1.2	1.3	1.3
A	1.3	1.2	1.5	1.5
LVdp / dtmin (kPa / s)				
B	385	307	276	251
A	367	297	280	270
τ relaxation (ms)				
B	31	31	29	36
A	32	31	33	39 [a]
AP (cmH_2O)				
B	14.8	15.7	17	18.3
A	14.9	15.7	17	18.3
LCXBF (ml / min)				
B	27	29	28	26
A	25	31	28	23
CCBF (ml / min)				
B	101	99	75	48
A	87 [a]	74 [a]	45 [a]	31 [a]

Median values ($n = 9$) before (B) and at peak effect after (A) the intravenous administration of increasing doses of sumatriptan at 30–45 min intervals. Abbreviations: see Table 1. [a] Significantly different from solvent.

Table 3
Haemodynamic and pulmonary parameters before (B) and after (A) the intravenous administration of ergotamine to anaesthetized dogs

Parameter	Ergotamine (μg/kg i.v.)			
	1.25	5	20	80
HR (beats / min)				
B	101	104	79	89
A	84	78 [a]	68 [a]	85 [a]
AoPs (kPa)				
B	19.1	19.9	19.9	17.6
A	19.1	20.2	20.1	18.8
AoPd (kPa)				
B	12	12	11.9	9.9
A	12.1	12.7	13.5 [a]	9.4
PaPs (kPa)				
B	3.5	3.7	3.9	3.7
A	3.5	3.8	3.9	3.9
PaPd (kPa)				
B	1.1	1.2	1.4	1.5
A	1.1	1.4	1.6 [a]	1.7 [a]
LVEDP (kPa)				
B	2.1	2	2.1	2
A	2.3	2.4	2.2	1.9
LVdp / dtmax (kPa / s)				
B	315	284	230	193
A	293	236 [a]	203 [a]	191 [a]
LVdp / dtmax / pd (/ s)				
B	39	37	32	29
A	37	29 [a]	28 [a]	27 [a]
SV (ml)				
B	18	19	19	15
A	17	20	12	11
CO (l / min)				
B	1.8	1.8	1.2	1.4
A	1.6	1.2	1.1 [a]	0.9 [a]
PRP (× 1000) (kPa × b / min)				
B	2.1	2.1	1.6	1.5
A	1.9	1.6 [a]	1.6	1.6
SVR (kPa / l × min)				
B	8.3	8.1	10.3	12.5
A	9.2	12.5 [a]	18.3	18
PVR (kPa / l × min)				
B	1	1	1.5	2.1
A	1	1.8 [a]	2.4	2.9
LVdp / dtmin (kPa / s)				
B	368	328	301	277
A	348	304	279	190
τ relaxation (ms)				
B	32	32	34	35
A	34	39	37	44 [a]
AP (cmH_2O)				
B	13.4	14.5	15.1	16
A	13.7	15.6 [a]	19.4 [a]	22.4 [a]
LCXBF (ml / min)				
B	20	20	13 [a]	14 [a]
A	21	18 [a]	12 [a]	13 [a]
CCBF (ml / min)				
B	100	86	46 [a]	39 [a]
A	79 [a]	45 [a]	27 [a]	21 [a]

Median values ($n = 7$) before (B) and at peak effect after (A) the i.v. administration of increasing doses of ergotamine at 30–45 min intervals. Abbreviations: see Table 1. [a] Significantly different from solvent.

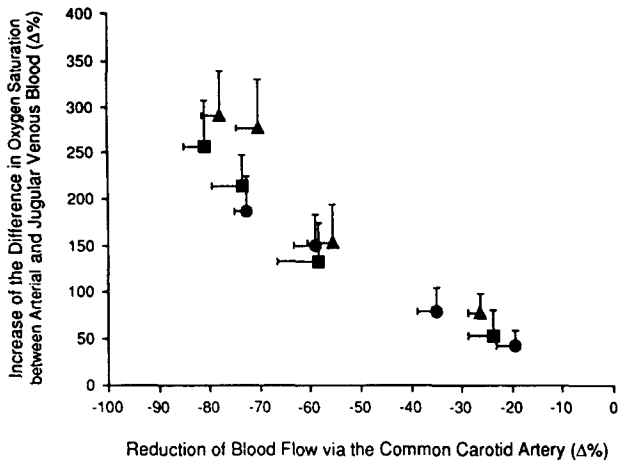


Fig. 3. Correlation between the reduction of carotid arterial blood flow and the increase of the difference in oxygen saturation between arterial and jugular venous blood elicited by alniditan, sumatriptan and ergotamine in anaesthetized dogs. Percentages of change versus premedication values of common carotid blood flow and of the difference in oxygen saturation between arterial and jugular venous blood elicted by alniditan (▲; $n = 9$), sumatriptan (●; $n = 9$) and ergotamine (■; $n = 7$). Means + S.E.M. The compounds were administered i.v. in increasing doses (right to left) of 1.25, 5, 20 and 80 μg/kg. Relative to solvent (data not shown), alniditan, sumatriptan and ergotamine elicit a dose-related increase of the difference in oxygen saturation between arterial and jugular venous blood. Changes elicited in the two parameters by the compounds are inversely correlated within each medication group (Pearson correlation coefficient $r = -0.96$ for alniditan; $r = -0.99$ with sumatriptan; $r = -0.97$ for ergotamine).

coefficient $r = 0.94$ for alniditan; $r = 0.94$ for sumatriptan; $r = 0.99$ for ergotamine).

3.3. Regional haemodynamic effects

In comparison with solvent, alniditan as well as sumatriptan (1.25–80 μg/kg i.v.) significantly reduced common carotid artery blood flow from 1.25 μg/kg i.v. on. Again, the extent of common carotid artery blood flow reductions was more pronounced with alniditan at 1.25 and 5 μg/kg i.v. than with similar doses of sumatriptan (Table 4). Relative to solvent, neither alniditan nor sumatriptan produced consistent changes in the duration of the PQ-, QRS-, QT- or QTc-intervals of the ECG; the incidences of changes in the amplitude of the ST-segment and the T-wave of the ECG relative to premedication values did not differ significantly between solvent, alniditan and sumatriptan (results not shown).

In comparison with solvent, neither alniditan nor sumatriptan (1.25–80 μg/kg i.v.) elicited significant changes in superior mesenteric artery blood flow (Table 4). Both alniditan and sumatriptan (1.25–80 μg/kg i.v.) produced a trend to a dose-related renal artery blood flow reduction, not reaching statistical significance in comparison with solvent; the extent of renal artery blood flow reductions varied considerably between individual animals. The changes in renal artery blood flow elicited by alniditan were not significantly different from those induced by sumatriptan. The reduction of common carotid artery blood flow elicited by alniditan (1.25, 5, 20 μg/kg i.v.) or sumatriptan (1.25, 5, 80 μg/kg i.v.) was significantly more pronounced than that of renal artery blood flow in the same preparations (Table 4).

3.4. Duration of action

In comparison with solvent (data not shown), alniditan at 5 μg/kg i.v. significantly reduced common carotid artery blood flow from a premedication control level of 116.8 ± 19.7 ml/min ($n = 5$) to a lowest value of 58.2 ± 11.6 ml/min (−50.1%) at 5 min after medication without producing significant changes throughout the 210 min

Table 4
Effect of solvent, alniditan and sumatriptan on common carotid, superior mesenteric and renal arterial blood flow in anaesthetized dogs

Parameter [a]	% Change at dose (μg/kg i.v.)			
	1.25	5	20	80
CCBF				
Solvent	3 ± 2	−4 ± 5	−9 ± 7	−11 ± 7
Alniditan	−39 ± 5 [b,c]	−56 ± 4 [b,c]	−63 ± 4 [b]	−65 ± 4 [b]
Sumatriptan	−12 ± 3 [b]	−23 ± 6 [b]	−23 ± 14 [b]	−48 ± 11 [b]
MBF				
Solvent	2 ± 4	9 ± 10	2 ± 9	3 ± 16
Alniditan	2 ± 3	5 ± 4	15 ± 4	27 ± 9
Sumatriptan	1 ± 5	−7 ± 5	−4 ± 9	5 ± 7
RBF				
Solvent	1 ± 1	4 ± 11	9 ± 5	11 ± 16
Alniditan	−6 ± 3	−16 ± 8	−34 ± 9	−37 ± 13
Sumatriptan	1 ± 1	0 ± 4	−11 ± 3	−14 ± 14

[a] Percentage peak change of common carotid (CCBF), superior mesenteric (MBF) and renal (RBF) arterial blood flow versus premedication values after the i.v. administration of solvent ($n = 7$), alniditan ($n = 8$) and sumatriptan ($n = 10$) at 45 min intervals. [b] Significantly different from solvent. [c] Significantly different from sumatriptan.

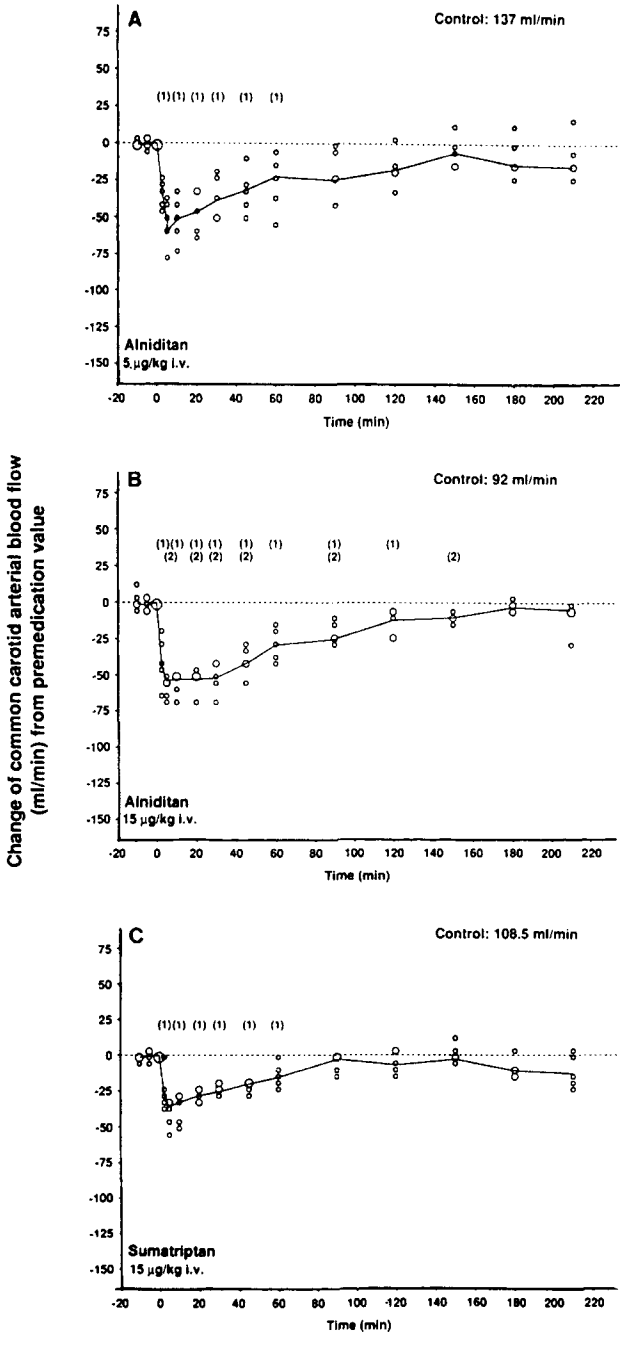


Fig. 4. Duration of action of alniditan and sumatriptan on carotid arterial blood flow in anaesthetized dogs. Change (in ml/min) versus premedication values (control: median value in ml/min) of the common carotid artery blood flow induced by alniditan (A: 5 µg/kg i.v., $n = 5$; B: 15 µg/kg i.v., $n = 5$) and sumatriptan (C: 15 µg/kg i.v., $n = 5$). Median value (---) and individual data (○). Relative to solvent (data not shown), alniditan at 5 and 15 µg/kg i.v. elicits a fast, protracted and dose-dependent reduction of common carotid artery blood flow. The extent and duration of that effect elicited by the former compound at 5 µg/kg i.v. is similar to the changes produced by a 3-fold higher dose of sumatriptan (15 µg/kg i.v.). The reduction of common carotid artery blood flow induced by alniditan at 15 µg/kg i.v. is significantly more pronounced than that observed after the same dose of sumatriptan. (1) Significantly different from solvent. (2) Significantly different from sumatriptan.

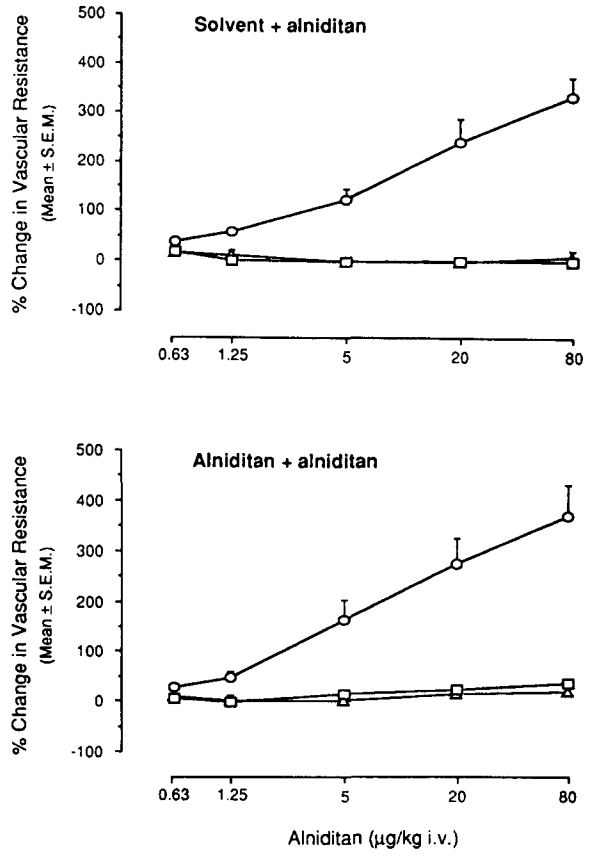


Fig. 5. Comparative effects of repeated medication with alniditan on carotid arterial vascular resistance, coronary arterial vascular resistance and systemic vascular resistance in anaesthetized dogs. Percentage of change versus premedication values of common carotid arterial vascular resistance (○), coronary arterial vascular resistance (△) and systemic vascular resistance (□) induced by solvent i.v. and by alniditan (0.63–80 µg/kg i.v.) in dogs premedicated daily for 4 days with solvent i.v. (solvent + solvent, $n = 6$; solvent + alniditan, $n = 6$) or alniditan 20 µg/kg i.v. (alniditan + alniditan, $n = 8$). Means + S.E.M. Relative to solvent (data not shown; $n = 6$), alniditan (0.63–80 µg/kg i.v.) elicits a significant and dose-related increase in carotid arterial vascular resistance without relevant effects on coronary arterial and systemic vascular resistance in anaesthetized dogs premedicated 4 times with solvent i.v. or alniditan (20 µg/kg i.v. daily for 4 days). The increase of carotid arterial vascular resistance produced by increasing doses of alniditan is similar after repeated premedication with solvent or with alniditan.

observation period in heart rate, systolic and diastolic aortic pressure and ECG characteristics. Relative to solvent, common carotid artery blood flow remained significantly reduced for 60 min after medication (Fig. 4).

At a dose of 15 µg/kg i.v., the compound significantly reduced common carotid artery blood flow from a premedication control value of 97.4 ± 7.28 ml/min ($n = 5$) to a lowest level of 36.8 ± 5.26 ml/min (−62.2%) at 5 min after medication. Common carotid artery blood flow remained significantly lower than that in the solvent-treated group for 120 min after medication (Fig. 4); in comparison with solvent, no significant changes occurred in heart rate, systolic and diastolic aortic pressure as well as in ECG

characteristics throughout the 210 min observation period (results not shown).

In comparison with solvent, sumatriptan at 15 μg/kg i.v. significantly reduced common carotid artery blood flow from a premedication control level of 102.8 ± 8.7 ml/min ($n = 5$) to a lowest value of 60.2 ± 8.7 ml/min (−41.4%) at 5 min after medication. Common carotid artery blood flow remained significantly lower than that in the solvent-treated group during 60 min after medication (Fig. 4); relative to solvent, no significant changes occurred in heart rate, systolic and diastolic aortic pressure as well as in ECG characteristics throughout the 210 min observation period (results not shown).

The extent of common carotid artery blood flow reductions elicited by alniditan at 5 μg/kg i.v. were not significantly different at any time period throughout the 210 min observation period from those produced by sumatriptan at 15 μg/kg i.v. By contrast, the reductions of common carotid artery blood flow elicited by alniditan at a dose of 15 μg/kg i.v. were significantly more pronounced than those produced by sumatriptan at 15 μg/kg i.v. for up to 150 min after medication (Fig. 4).

3.5. Repeated medication

Relative to solvent ($n = 6$), alniditan (0.63–80 μg/kg i.v.) elicited a significant and dose-related increase in common carotid arterial vascular resistance to flow without relevant effects on coronary arterial vascular resistance, systemic or pulmonary vascular resistance, heart rate, systolic or diastolic aortic pressure in anaesthetized dogs premedicated either with solvent i.v. (daily for 4 days; $n = 6$) or with alniditan (20 μg/kg i.v. daily for 4 days; $n = 8$). The augmentations of carotid arterial vascular resistance elicited by increasing doses of alniditan were similar after repeated premedication with solvent or alniditan (Fig. 5).

4. Discussion

The present study demonstrates that the intravenous administration of alniditan to anaesthetized dogs elicits a pronounced, dose-related reduction of common carotid arterial blood flow and an increase of the difference in oxygen saturation between arterial and jugular venous blood. At doses eliciting such effects, the compound produces little or no changes in coronary blood flow and resistance to flow, mesenteric and renal arterial blood flow, systemic and pulmonary vascular resistance, airway resistance, blood pressure, heart rate, cardiac contractile performance and relaxation and ECG characteristics. In the absence of significant changes in blood pressure, the reduction of carotid arterial blood flow by alniditan is accompanied by an increase of vascular resistance in that vascular bed. This indicates that alniditan elicits a comparatively selective vasoconstriction within the carotid vascular region. Similar to sumatriptan in vitro, alniditan constricts isolated large intracranial vessels, in particular porcine and canine basilar arteries and canine middle cerebral arteries, with little or no effect on other arteries (W. Janssens, personal communication).

However, a constriction of large cerebral conducting arteries via 5-HT_1-like receptor activation with sumatriptan does not change cerebral capillary blood flow (Perren et al., 1989; Den Boer et al., 1992a, b; Friberg et al., 1991). It is therefore unlikely that the former effect would be the mechanism by which alniditan reduces blood flow via the common carotid artery. Using radioactive microspheres of 10–35 μm diameter, it has been shown that, in dogs, only 2–3% of the carotid blood flow is distributed to the brain, whereas 40–50% is shunted to the venous side via arteriovenous anastomoses, localized in extracerebral structures, e.g. head skin, ears, tongue and nasal mucosa (Saxena, 1986; Saxena et al., 1983; Johnston and Saxena, 1978). Reduction of shunt blood flow via such anastomoses correlates with the increase of the difference in oxygen saturation between arterial and jugular venous blood (Saxena et al., 1983; Johnston and Saxena, 1978; Schamhardt et al., 1979). The dose-dependent increase of the arteriovenous difference in oxygen saturation by alniditan thus suggests that the compound reduces common carotid arterial blood flow and increases resistance in that vascular region by a constriction of cranial arteriovenous anastomoses (Feniuk et al., 1989; Saxena et al., 1983; Villalon et al., 1992). In this respect, alniditan has a fast onset of action and a protracted effect at low doses devoid of substantial repercussions on other vascular areas.

Moreover, tolerance or resetting of sensitivity to alniditan does not occur since repeated premedication with the compound does not modify its effect on common carotid blood flow and resistance to flow in that vascular bed. Also similar to sumatriptan in vivo (this study; Perren et al., 1989; Cambridge et al., 1992), alniditan produces a trend for transient renal arterial blood flow reduction, the extent of which is significantly smaller than that of common carotid arterial blood flow reductions in the same preparation. Such a transient and modest reduction by sumatriptan of renal arterial blood flow may be due to an activation of 5-HT_1-like receptors causing vasocontriction, counter-balanced by vasodilatation elicited by 5-HT_1-like receptor-mediated release of nitric oxide in canine renal vasculature (Cambridge et al., 1992; Takahashi et al., 1992). The vasoconstrictive action of alniditan on arteriovenous anastomoses in the carotid vascular region thus resembles that of antimigraine compounds such as sumatriptan, ergotamine and methysergide (Feniuk et al., 1989; Den Boer et al., 1991; Saxena et al., 1983; Johnston and Saxena, 1978; Saxena, 1974). Although the profile of activity of alniditan resembles that of sumatriptan, the former is approximately 3 times as potent on a μg/kg i.v. basis than the latter in reducing carotid arterial perfusion.

Whether or not the longer duration of action of alniditan we observed in comparison with sumatriptan, administered in equipotent intravenous doses to anaesthetized dogs, persists in man obviously depends e.g. on the routes of administration used for therapeutic purposes in patients as well as on potential species differences in metabolic handling and in sensitivity of relevant blood vessels to the effects of both compounds.

In contrast to alniditan and sumatriptan, ergotamine induces also marked increases in systemic and pulmonary vascular resistance and in airway resistance, attenuates coronary arterial perfusion and causes bradycardia at doses increasing vascular resistance in the carotid vasculature (this study; Feniuk et al., 1989; Saxena et al., 1983; Johnston and Saxena, 1978; Villalon et al., 1992).

In the present experimental conditions, we found no indication that alniditan causes vasodilatation in vivo. Such a dilatation via an agonistic effect at prejunctional 5-HT_1-like receptors, reducing release of mediators from nerve endings as documented in vitro has been described for low doses of sumatriptan (Feniuk et al., 1989) and methysergide (Feniuk et al., 1981) in the canine femoral arterial circulation which contains arteriovenous anastomoses in skin and food pads (Hales, 1974; Spence et al., 1972; Delaney and Scarpino, 1973). However, the balance in favour of dilatation or constriction depended largely upon the status of sympathetic activity of the preparation (Feniuk et al., 1981). Thus, it remains to be examined whether alniditan can produce dilatation of particular vascular beds in conditions of a sympathetic nerve activity different from the one achieved in the present experiments.

In conclusion, this study demonstrates that alniditan, at low intravenous doses with little or no overall haemodynamic and pulmonary effects, increases carotid vascular resistance, probably due to reduction of extracerebral shunt flow following a constriction of cephalic arteriovenous anastomoses in anaesthetized dogs. Whether or not an effect on 5-HT_1-like receptors similar to the ones activated by sumatriptan (Feniuk et al., 1989; Perren et al., 1989, 1991; Den Boer et al., 1992a, b) is involved in the selective carotid arterial vasoconstriction elicited by alniditan remains to be elucidated.

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References

Cambridge, D., L.J. Butterfield and H.V. Whiting, 1992, Sumatriptan stimulates nitric oxide release in vivo, in: 2nd International Symposium on Serotonin from Cell Biology to Pharmacology and Therapeutics, Houston, USA, September 15–18, Abstract p. 20.

Delaney, J. and J. Scarpino, 1973, Limb arteriovenous shunting following sympathetic denervation, Surgery 73, 202.

Den Boer, M.O., C.M. Villalon, J.P.C. Heiligers, P.P.A. Humphrey and P.R. Saxena, 1991, Role of 5-HT_1-like receptors in the reduction of porcine cranial arteriovenous anastomotic shunting by sumatriptan, Br. J. Pharmacol. 102, 323.

Den Boer, M.O., J.A.E. Somers and P.R. Saxena, 1992a, Lack of effect of the antimigraine drugs, sumatriptan, ergotamine and dihydroergotamine on arteriovenous anastomotic shunting in the dura mater of the pig, Br. J. Pharmacol. 107, 577.

Den Boer, M.O., C.M. Villalon and P.R. Saxena, 1992b, 5-HT_1-like receptor mediated changes in porcine carotid haemodynamics: are 5-HT_{1D} receptors involved?, Naunyn-Schmied. Arch. Pharmacol. 345, 509.

Feniuk, W., P.P.A. Humphrey and A.D. Watts, 1981, Modification of the vasomotor actions of methysergide in the femoral bed of the anaesthetized dog by changes in sympathetic nerve activity, J. Auton. Pharmacol. 1, 127.

Feniuk, W., P.P.A. Humphrey and M.J. Perren, 1989, The selective carotid arterial vasoconstrictor action of GR43175 in anaesthetized dogs, Br. J. Pharmacol. 96, 83.

Feniuk, W., P.P.A. Humphrey, M.J. Perren, H.E. Connor and E.T. Whalley, 1991, Rationale for the use of 5-HT_1-like agonists in the treatment of migraine, J. Neurol. 238, S38.

Friberg, L., J. Olesen, H.K. Iversen and B. Sperling, 1991, Migraine pain associated with middle cerebral dilatation: reversal by sumatriptan, Lancet 338, 13.

Hales, J.R.S., 1974, Radioactive microsphere techniques for the studies of the circulation, Clin. Exp. Pharmacol. Physiol. Suppl. 1, 31.

Humphrey, P.A., W. Feniuk, M.J. Perren, H.E. Connor and A.W. Oxford, 1989, The pharmacology of the novel 5HT_1-like receptor agonist, GR43175, Cephalalgia 9 (Suppl. 9), 23.

Johnston, B.M. and P.R. Saxena, 1978, The effect of ergotamine on tissue blood flow and the arteriovenous shunting of radioactive microspheres in the head, Br. J. Pharmacol. 63, 541.

Milliken, G.A. and D.E. Johnson (eds.), 1984, in: Analysis of Messy Data (Wadsworth, Belmont, CA) p. 473.

Perren, M.J., W. Feniuk and P.P.A. Humphrey, 1989, The selective closure of feline carotid arteriovenous anastomoses (AVAs) by GR43175, Cephalalgia 9 (Suppl. 9), 41.

Perren, M.J., W. Feniuk and P.P.A. Humphrey, 1991, Vascular 5-HT_1-like receptors that mediate contraction of the dog isolated saphenous vein and carotid arterial vasoconstriction in anaesthetized dogs are not of the 5-HT_{1A} or 5-HT_{1D} subtype, Br. J. Pharmacol. 102, 191.

Saxena, P.R., 1974, Selective vasoconstriction in carotid vascular bed by methysergide: possible relevance to its antimigraine effect, Eur. J. Pharmacol. 27, 99.

Saxena, P.R., 1986, Pharmacology of cranial arteriovenous anastomoses, in: Neuronal Regulation of Brain Circulation, eds. C. Owman and J.E. Hardebo (Elsevier, Amsterdam) p. 541.

Saxena, P.R. and M.O. Den Boer, 1991, Pharmacology of antimigraine drugs, J. Neurol. 238, S28.

Saxena, P.R. and P.D. Verdouw, 1982, Redistribution by 5-hydroxytryptamine of carotid arterial blood at the expense of arteriovenous anastomotic blood flow, J. Physiol. 332, 501.

Saxena, P.R., N.A. Koedam, J. Heiligers and R.P. Hof, 1983, Ergotamine-induced constriction of cranial arteriovenous anastomes in dogs pretreated with phentolamine and pizotifen, Cephalalgia 3, 71.

Schamhardt, H.C., P.D. Verdouw, T.M. van der Hoek and P.R. Saxena, 1979, Regional myocardial perfusion and wall thickness and arteriovenous shunting after ergotamine administration to pigs with a fixed coronary stenosis, J. Cardiovasc. Pharmacol. 1, 673.

Siegel, S., 1956, Nonparametric Statistics for the Behavioral Sciences (McGraw-Hill Kogakusha, Tokyo) p. 96, p. 116.

Spence, R.J., B.A. Rhodes and H.N. Wagner, 1972, Regulation of

arteriovenous anastomotic and capillary blood flow in the dog leg, Am. J. Physiol. 222, 326.

Takahashi, T., J. Hisa and S. Satoh, 1992, Serotonin-induced vasoconstriction in dog kidney, J. Cardiovasc. Pharmacol. 20, 779.

Thompson, D.S., C.B. Waldron, S.M. Juul, N. Naqvi, R.H. Swanton, D.J. Coltart, B.S. Jenkins and M.M. Webb-Peploe, 1982, Analysis of left ventricular pressure during isovolumic relaxation in coronary artery disease, Circulation 65, 690.

Van de Water, A., R. Xhonneux, R.S. Reneman and P.A.J. Janssen, 1992, Cardiac and hemodynamic effects of intravenous R 80 122, a new phosphodiesterase III inhibitor, in a canine model of myocardial ischemia and heart failure, J. Cardiovasc. Pharmacol. 20, 18.

Villalon, C.M., A.H. Bom, M.O. Den Boer, J.P.C. Heiligers and P.R. Saxena, 1992, Effects of S9977 and dihydroergotamine in an animal experimental model for migraine, Pharmacol. Res. 25, 125.