Subarachnoid hemorrhage induced sympathoexcitation arises due to changes in endothelin and/or nitric oxide activity

Elisabeth Lambert*, Gavin Lambert, Céline Fassot, Peter Friberg, Jean-Luc Elghozi

*Laboratoire de Pharmacologie, CNRS UMR 8604, Faculté de Médecine Necker, 156 rue de Vaugirard, 75015 Paris, France
bDepartment of Clinical Physiology, Sahlgrenska University Hospital, S-413 45 Göteborg, Sweden

Received 14 September 1999; accepted 26 November 1999

Abstract

Objective: The demonstration of the effectiveness of endothelin antagonists and nitric oxide donors in managing vasospasm following subarachnoid hemorrhage is encouraging. Whether such drugs can modify the sympathoexcitation that accompanies this condition remains unknown and was the basis for the present report. Methods: Subarachnoid hemorrhage was induced in conscious rats by injecting blood via a catheter placed along the surface of the brain and directed towards the circle of Willis. We combined measurements of arterial plasma catecholamines with the spectral analysis of blood pressure variability in order to examine sympathetic nervous activation following subarachnoid hemorrhage. Experiments were performed in untreated animals and in rats following pretreatment with either bosentan or sodium nitroprusside. Results: Indicative of a pronounced sympathoexcitation, the 0.2–0.6 Hz frequency components of blood pressure were markedly elevated following subarachnoid hemorrhage (2.5±0.6 vs. 8.9±2.6 mmHg, P<0.01). Parallel changes in plasma norepinephrine concentration were observed (1.0±0.2 vs. 2.4±0.4 nmol/l, P<0.01). The subarachnoid injection of saline did not modify blood pressure variability or plasma norepinephrine concentrations. Pretreatment with either bosentan or sodium nitroprusside completely prevented the subarachnoid hemorrhage induced sympathoexcitation. Conclusions: Experimental subarachnoid hemorrhage is associated with a pronounced activation of the sympathetic nervous system. It would appear that this sympathoexcitation has its roots ensconced in either the release of endothelin or an impairment in nitric oxide mediated vasodilation.

Keywords: Autonomic nervous system; Blood pressure; Cerebrovascular disorder; Endothelins; Nitric oxide

1. Introduction

Treatment of subarachnoid hemorrhage (SAH) remains one of the major challenges in neurosurgery. Despite improved diagnosis and treatment there remains a substantial morbidity and mortality associated with this condition. Some studies suggest that elevated levels of catecholamines [1], coupled with an abnormal sensitivity of the cerebral vasculature to these catecholamines [2], may be involved in the genesis of cerebral vasospasm, predisposing patients to cerebral ischaemia, and potentially life-threatening arrhythmias. Indeed, the prophylactic use of calcium antagonists are routinely used in order to limit the consequences of cerebral vasospasm [3]. Although not yet subjected to rigorous clinical trial, the recent demonstration of the effectiveness of endothelin antagonists [4,5] and nitric oxide (NO) donors [4] in managing vasospasm following experimental and clinical SAH [6] holds further promise in the treatment of this condition. Whether such agents are able to limit the numerous systemic complications associated with this condition remains problematic.

Recent evidence implicates the sympathetic nervous system’s involvement in the genesis of the systemic perturbations seen in patients following non-traumatic SAH [7]. Indeed, cardiac complications are common following SAH [8,9] and accumulating evidence suggests that ECG-abnormalities reflect underlying cardiac pathol-
ogy and dysfunction [10]. The improved autonomic function and survival in SAH patients treated with metoprolol provides further support that the sympathoexcitation accompanying SAH is not benign [11].

In the series of experiments presented in this report we adapted an existing technique [12,13] in order to examine the acute effects of experimentally induced subarachnoid bleeding in conscious, freely moving animals. We combined measurements of arterial plasma catechol concentrations with the spectral analysis of blood pressure variability in order to examine the hemodynamic and sympathetically mediated sequelae following experimentally induced SAH. The rationale for using power spectral analysis of circulatory rhythms as an index of sympathetic function stems from the demonstration that combined \( \alpha \) and \( \beta \)-adrenoreceptor blockade, or destruction of sympathetic nerves, results in elimination of spectral power of blood pressure at frequencies around 0.4 Hz in rats [14]. A direct coupling between renal sympathetic nerve activity and oscillations in arterial blood pressure at 0.4 Hz has also been demonstrated [15].

Previous reports have demonstrated the occurrence of cerebral vasospasm following the subarachnoid injection of blood [16]. In order to examine whether such events could have their roots ensconced in an activation of the sympathetic nervous system we performed experiments in untreated animals and, subsequently, in rats following pretreatment with the endothelin antagonist bosentan. In a further series of experiments, SAH was induced against a background infusion of a low dose NO donor, namely sodium nitroprusside.

2. Methods

Experiments were performed on 39 male Wistar rats (Janvier, Le Genest-Saint-Isle, France), weighing between 275–350 g. Throughout the course of the experiment animals were kept in a constant light–dark cycle (light 0800–2000 h), in a room maintained at 24°C with a relative humidity of 50–60%. All experiments conformed with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996) as well as to the relevant guidelines of the French Ministry of Agriculture for scientific experimentation on animals. Our laboratory and personnel are authorized to conduct such investigations according to the Ministry’s Executive Order No 89-02683.

2.1. Animal preparation

At least 3 days prior to the experimental day, animals were surgically implanted with a catheter in the subarachnoid space following anesthesia with sodium pentobarbital (60 mg/kg i.p., Sanofi, Libourne, France). The catheter was constructed on site using 30 mm of Silastic tubing \((0.30 \times 0.64 \text{ mm, Dow Corning, Midland, Michigan, USA})\) and 50 mm of \(0.58 \times 0.96\) polyethylene tubing (Biotrol, Paris, France). The two pieces of tubing were connected using Vetbond tissue adhesive (3M, St. Paul, Germany) and the catheter’s patency and integrity checked prior to usage with sterile saline. The narrow bore end of the catheter was subsequently trimmed to a final length of 15 mm. Animals were placed on a small-animal stereotaxic instrument, and the incisor bar was adjusted until the heights of lambda and bregma skull points were equal. This flat-skull position was achieved when the incisor bar was lowered 3.3±0.4 mm below horizontal zero. The skull was exposed via a 30-mm midline scalp incision and a small bore hole was drilled in the left lateral frontal bone 10 mm anterior to the intra-aural line and 3.4 mm lateral to the mid-sagittal line. The dural membrane was pierced with a sterile, 26G needle and the narrow bore, 15-mm portion of the intra-cranial catheter was very carefully guided along the left lateral surface of the brain to the base of the skull and directed posteriomedially towards the circle of Willis. The catheter was anchored and the burr hole in the skull sealed using a zinc oxide eugenol based temporary crown and bridge cement (Kerr, Turin, Italy). The catheter was sealed using a hardened steel pin and tunnelled under the skin and exteriorized via an incision in the neck. Animals were treated with penicillin (100 000 I.U., i.p., Diamant, Puteaux, France) and their temperature maintained at 37°C throughout their recovery from the anesthesia and surgery. Upon regaining consciousness, animals were housed in individual cages of identical dimensions with free access to standard rat chow and water.

2.2. Experimental preparation

Experiments were performed on unrestrained, fully conscious animals. The arterial catheter was connected to a pressure transducer (Spectramed P10EZ, Bihlloven, The Netherlands) linked with a Gould RS 3400 polygraph (Ballainvilliers, France) in order to monitor pulsatile blood pressure. The output from the blood pressure preamplifier was connected to an A/D converter to permit data acquisition, storage and analysis using a 486 DX computer from Fujikama (Toronto, Canada). Where required, the rat’s venous catheter was connected to a microsyringe for drug or saline injections. Resting hemodynamic recordings were commenced at least 30 min after the animals had been connected to the pressure transducer. The blood pressure signal was digitized using a 12-bit A/D converter.
at a rate of 500 Hz and processed by an algorithm based on feature extraction to detect and measure the characteristics of a blood pressure cycle with its maximum in a 100-ms window (Notocord Systems, Igny, France). These windows were adjusted to the heart rate of the rats and allowed acquisition of a rate up to 600 bpm. The systolic blood pressure (SBP), the preceding diastolic pressure (DBP), minimum and heart rate calculated as 60 000/heart period (ms) measured from the SBP present in the 100-ms window and the preceding upstroke were stored on computer disk. No interpolation was performed. The evenly spaced (equidistant) sampling allowed a direct spectral analysis using a Fast Fourier transform algorithm on 1024 point time series of a stationary period. Stationary periods corresponded to segments with a single narrow respiratory component. This corresponded to a 102.4-s period. Thus each spectral component (band) corresponded to a harmonic of 10 000/1024 mHz i.e., 0.00977 Hz. The first spectral component (0–0.00977 Hz) corresponded to the continuous part of the signal. The frequency of oscillations scale (abscissa) was analyzed up to 2.5 Hz. Power of the heart rate or blood pressure spectrum (ordinates) had units of bpm^2 or mmHg^2. The sum of the values of consecutive bands was calculated to represent an integrated spectrum over a predetermined frequency range. Integrated spectra of the systolic blood pressure, diastolic blood pressure and heart rate were computed in the mid (0.2–0.6 Hz, MF) frequency band. Simple statistics, comprising the mean and standard deviation (S.D.) of the distribution of the variables of the 102.4-s files (1024 values) used for the spectral analysis were computed.

2.3. Experimental protocol

Resting hemodynamic measurements were commenced at 10:00 and generally comprised a 30-min recording period; afterwards a 1-ml arterial blood sample for catecholamine analysis was obtained. Blood was withdrawn slowly using a 1-ml syringe and immediately transferred into a chilled tube containing an anticoagulant–antioxidant mix containing ethyleneglycol and reduced glutathione, centrifuged and the plasma separated and stored at −80°C for subsequent analysis. The red blood cells were reconstituted in artificial plasma (Plasmion, Laboratoire Roger Bellon, Neuilly Sur Seine, France) and reinjected into the animals. A 200-µl portion of homologous, arterial blood was then withdrawn from the arterial catheter and injected slowly over a period of 1 min into the subarachnoid space via the intracerebral catheter using a 250-µl glass Hamilton syringe with 22-gauge needle (no. 725, Hamilton, Bonaduz, Switzerland). Hemodynamic parameters were continuously monitored for 5 h with the recordings being divided into six periods comprising: control and 0–1, 1–2, 2–3, 3–4 and 4–5 h post-hemorrhage. Each period consisted of the average of between 5 and 10, 102.4-s artifact-free segments of blood pressure and heart rate monitoring. The 5–10 artifact-free segments for the analysis of blood pressure and heart variabilities were selected by visual inspection of the raw blood pressure signal. The segments taken were representative of the entire signal. Following the last period of recording a blood sample for plasma catechol analysis was obtained. At the completion of all experimental protocols animals were killed by overdose with pentobarbitone and the position of the intracerebral catheter and subsequent hematoma was confirmed by visual inspection. Data obtained from animals where there was evidence of catheter-induced brain trauma or hemorrhage within the brain structure are not included in this analysis (n=4).

2.4. Experimental groups

Animals were divided into five experimental groups. In group 1 (n=7) the effects of acute experimental SAH were examined. Animals did not receive any pharmacological intervention prior to or subsequent to the induction of SAH. Rats in group 2 (n=7) received saline instead of blood in the subarachnoid space in order to examine the influence of increased intracranial pressure per se. In the third experimental group (n=11), the effectiveness of endothelin blockade, initiated just prior to the subarachnoid injury, was assessed by examining the effects of blood injected into the subarachnoid space 20 min after the intravenous administration of bosentan (5 mg/kg). To ensure the integrity of endothelin receptor blockade throughout the duration of the experiment a second injection of bosentan (3 mg/kg, i.v.) was given 2.5 h after induction of the subarachnoid bleeding. In order to eliminate the possible confounding effect of blood pressure reduction following acute bosentan we incorporated another group of animals (group 4, n=8) whereby the effects of experimentally induced SAH were examined after prior blockade of endothelin release by a 5-day pretreatment with bosentan. Immediately after the animal’s recovery following the surgical placement of the subarachnoid and arterial catheters, animals were given 50 mg/kg i.p. bosentan and then subsequently over the following 5 days. Experimentation was commenced 1 h after the bosentan injection on the fifth day. In all examinations involving bosentan, the integrity of blockade of endothelin receptors was assessed from the effect of 0.4 nmol/kg i.v. endothelin administration on blood pressure prior to and at the completion of the experimental period. In the fifth group of animals (n=6) SAH was induced against a background intravenous infusion of the NO donor, sodium nitroprusside (50 µg/ml infused at a rate of 0.36 ml/h). Blood samples for catecholamine analysis were not obtained in this group of animals.

2.5. Catechol measurements

Catechols were extracted from plasma with alumina adsorption, separated by high performance liquid chromatography and the amounts quantified by electrochemical
detection according to previously described methods [17]. The chromatographic system consisted of a Model 480 high precision pump, Model Gina autosampler, Model STH 585 column oven, Chromel 3.03 chromatography data system (Gynkotek, Germering, Germany), Model 5100A coulometric detector equipped with a Model 5021 conditioning cell and a Model 5011 analytical cell (Environmental Sciences Associates, MA, USA) and a 25-cm Altex Ultrasphere column (ODS 25 cm×4.6 mm, 5 μm particle size, Beckman Instruments, CA, USA, USA). Analysis was performed at 24°C with the operating potentials set at +0.35 V for the guard cell and −0.35 and +0.29 V for detectors 1 and 2, respectively. All measurements were made using the oxidizing potential applied at detector 2 and compounds in plasma were identified by their retention behavior compared to that of authentic standard solutions. The intra-assay coefficients of variation were ±2% for norepinephrine, ±10% for epinephrine, and ±2% for the intraneuronally produced metabolite of norepinephrine, dihydroxyphenylglycol (DHPG). All samples were analyzed in a single batch analysis.

2.6. Statistics

Values of blood pressure, heart rate and oscillations in blood pressure at 0.2–0.6 Hz are expressed as mean±standard error of the mean (S.E.M.). For examination of the effects of experimentally induced SAH on blood pressure and its variability, and heart rate, a one-way analysis of variance was used. Comparisons were made by orthogonal partitioning which compared the effects of blood or saline injection into the subarachnoid space at the various time intervals to that of the control period. The comparison between arterial catecholamines was made using Student’s t-test.

3. Results

3.1. Effect of injection of blood/saline on blood pressure, heart rate, blood pressure and heart rate variability and plasma catechol concentrations

Indicative of a pronounced sympathoexcitation, the oscillations in blood pressure at 0.2–0.6 Hz were elevated 2 h following the subarachnoid injection of blood (Fig. 1). This effect was maintained throughout the duration of the experimental period (Fig. 2). Whereas the elevation in blood pressure variability was not evident until 2 h after induction of the injury, the injection of blood into the subarachnoid space resulted in an immediate rise in blood pressure, peaking 1.5 h after the insult (Fig. 2). The elevation in systemic arterial pressure persisted for ~2.5 h and then tapered back to control values. Changes in heart

Fig. 1. Example of systolic and diastolic blood pressure recording and the corresponding systolic blood pressure spectrum in one untreated rat during control conditions (A) and 3 h after injection of blood into the subarachnoid space (B).
with no accompanying alteration in the resting blood pressure level. However, bosentan administered 2.5 h after the induction of SAH resulted in a reduction in blood pressure in the order of 15 mmHg (Fig. 3a). Blood pressure variability at 0.4 Hz was not altered in response to bosentan administration. Plasma concentrations of adrenaline and DHPG, but not norepinephrine, were elevated following bosentan administration (Table 1). Acute pretreatment with bosentan completely prevented the SAH induced increase in blood pressure, its variability and plasma catechol levels, however the associated elevation in heart rate persisted (Fig. 3a, Table 1).

3.1.2. Effect of chronic treatment with bosentan
Chronic treatment with bosentan was without any discernible effect on resting blood pressure and its variability and plasma norepinephrine and epinephrine levels, but elicited an increase in heart rate (338±9 vs. 396±16 bpm, P<0.01) and plasma DHPG (Table 1). In this series of experiments, the blood pressure rise observed following the subarachnoid injection of blood was completely prevented, in fact, there was a tendency for the blood pressure to be reduced by ~10 mmHg. The chronic administration of bosentan also prevented the rise in oscillations in blood pressure at 0.2–0.6 Hz, heart rate and plasma catechols following SAH (Fig. 3b, Table 1).

Blockade of endothelin receptors was confirmed by the lack of effect of 0.4 nmol/kg i.v. endothelin administration on blood pressure, both prior and at the completion of the experimental period (data unshown).

3.1.3. Effect of a low-dose infusion of sodium nitroprusside
Low dose infusion of sodium nitroprusside did not elicit any alterations in basal blood pressure, its variability or heart rate and also prevented SAH induced alterations in all these parameters (Fig. 4).

Table 1

| Arterial plasma catechol concentrations of epinephrine, norepinephrine and dihydroxyphenylglycol (DHPG) during control conditions and 5 h after injection of blood into the subarachnoid space in untreated and bosentan treated animals, and in rats following the subarachnoid injection of saline.a |
|-----------------|---------------|---------------|---------------|---------------|
|                  | Blood injection | Saline injection |               |               |
|                  | No treatment   | Acute bosentan | Chronic bosentan | No treatment   |
| Epinephrine      |               |               |               |               |
| Control          | 0.52±0.17     | 1.53±0.45*    | 1.08±0.40     | 0.53±0.28     |
| 5 h post SAH     | 1.86±0.4*     | 1.77±0.46     | 1.05±0.63     | 0.89±0.33     |
| Norepinephrine   |               |               |               |               |
| Control          | 1.05±0.21     | 1.48±0.34     | 1.64±0.28     | 0.84±0.11     |
| 5 h post SAH     | 2.43±0.36*    | 2.24±0.39     | 1.86±0.47     | 1.47±0.41     |
| DHPG             |               |               |               |               |
| Control          | 2.71±0.29     | 4.43±0.37*    | 3.95±0.48*    | 2.46±0.40     |
| 5 h post SAH     | 4.61±0.55*    | 4.34±0.51     | 3.83±0.42     | 3.64±0.91     |

* P<0.01, 5 h post SAH vs. control values; † P<0.05; ‡ P<0.01, vs. corresponding value in untreated animals.
demonstrated that experimentally induced SAH elicits an approximately three-fold increase in sympathetic nervous activation. Although we did not provide direct evidence of alterations in endothelin or NO activities, given the effectiveness of bosentan and sodium nitroprusside in preventing the acute rise in sympathetic nervous activity in our experiments, it would appear that the sympathoexcitation that we describe occurs secondary to a failure in the balance between endothelin and NO.

Sympathoexcitation, associated with elevations in systemic blood pressure, following non-traumatic SAH has been attributed to occur in response to the associated elevation in intracranial pressure. Cushing noted that controlled increments in cerebrospinal fluid pressure resulted in a marked blood pressure elevation [18]. Such a rise in blood pressure is prevented by \(\alpha\)-adrenergic blockade [19] and is believed to be mediated via alterations in neuronal activity in the brainstem [20,21], in response to local ischaemia [22], or more particularly, hypoxia [23]. It seems that marked elevations in arterial pressure occur only when the SAH induced rise in intracranial pressure approaches systemic blood pressure [24]. Although, not measured in the present report, Lorenzo et al. demonstrated a rise in intracranial pressure in the order of 50 mmHg 10 min after the subarachnoid injection of either saline, blood or blood fractions [13]. It would appear then that the rise in intracranial pressure generated in our animals rests well below systemic blood pressure and does not contribute extensively to the observed sympathoexcitation.

The observation that heart rate was increased immediately following the subarachnoid injection of blood raises the possibility that vagal mechanisms may be compromised by alterations in intracranial pressure. Interestingly, mid-frequency oscillations in heart rate may reflect the combined actions of the sympathetic nervous system and vagus nerve [25]. While further studies are required to unequivocally elucidate the vagaries of heart rate variability, our observation of unchanged mid-frequency components of heart rate variability in the face of pronounced sympathetic excitation raises the possibility that vagal activity be reduced following SAH. Indeed, combined sympathoexcitation and vagal withdrawal could account for the myriad of cardiovascular anomalies seen in patients following SAH. Clearly, further studies are required to delineate the role of the vagus in the generation of systemic complications following SAH.

4. Discussion

By combining a spectral analysis approach with measurements of plasma catechols in conscious rats, we have
origins in the form of constriction of vessels supplying the brainstem.

Our observations suggest that sympathoexcitation following SAH develops as a consequence of dysfunction in endothelial production and/or release of endothelin and NO. Endothelin is one of the most potent vasoconstricting compounds known. Endothelins propensity for vasoconstriction, coupled with its ability to sensitize blood vessels to other vasoactive compounds such as serotonin and norepinephrine [28], makes it a prime candidate in the development of cerebral vasospasm following SAH. Indeed, there exist numerous reports demonstrating the effectiveness of endothelin antagonists in preventing vasospasm following experimental SAH [4,5]. Moreover, there is now strong evidence indicating that SAH leads to decreased activity of NO in cerebral arteries [29]. Furthermore, perivascular hemoglobin and metabolite concentrations adjacent to the middle cerebral artery following experimental SAH are elevated 100-fold [30] and hemoglobin released after SAH is known to trap NO [31]. It would seem then that the sympathoexcitation we observe may have its roots ensconced in a shift in the balance between endothelin-induced contraction and NO mediated vasodilatation rather than endothelin stimulation per se. Such a view, as proposed by Sobey and Faraci [32], is not without precedent. Furthermore, NO donors have recently been demonstrated to be effective in limiting cerebral vasospasm following experimental SAH [4]. Moreover, brain topical superfusion of NO scavengers, such as hemoglobin, leads to the development of ischemic events with associated reductions in cerebral blood flow [33].

Given that in our experiments, both bosentan and sodium nitroprusside were administered peripherally, it is difficult to dissociate between the peripheral and central effects of either drug. On the basis of a recent clinical study it would appear that bosentan is not sympathoinhibitory [34]. Bosentan has been shown to gain access to the adventitial surface of feline pial resistance arterioles after systemic administration [35,36], and antagonize the vasoconstrictive effect of exogenous endothelin 1 [35]. This observation suggests that bosentan could act centrally. While there may well be sympathoinhibitory nitrergic neurons in the central nervous system, on balance, the usual effect of sodium nitroprusside, with attendant hypotension, is sympathetic nervous system activation. In our experiments, we used a dose of sodium nitroprusside that did not affect the blood pressure level, suggesting that there was probably little effect in the periphery.

Acknowledgements

This work was supported by grants from Fédération Française de Cardiologie, Fondation de France, and from the Swedish Medical Council. Gavin Lambert was supported initially by a CJ Martin Fellowship from the National Health and Medical Research Council of Australia and subsequently by a grant from Association Claude Bernard, France.

References

[18] Cushing H. Some experimental and clinical observations concerning