Most volatile or i.v. general anaesthetics alter cardiovascular function, often through endothelial-dependent mechanisms. They also have profound effects on plasma membrane properties. Caveolae are specialized subdomains of the plasma membrane with a distinct lipid and protein composition, which play an essential role in the physiology of endothelial cells (ECs). At present, it is unknown whether anaesthetics affect caveolae. However, accumulating evidence obtained either in caveolae research or in anaesthesia research, suggests that caveolae might be perturbed by volatile anaesthetics. This editorial proposes that the endothelium-dependent effects of anaesthetics on the cardiovascular system may be caveolae-mediated, and presents evidence to support this novel model.

Inhaled and i.v. general anaesthetics produce drug-specific circulatory effects, resulting in changes in systemic vascular resistance, systemic blood pressure, heart rate, cardiac output, stroke volume, right atrial pressure and coronary blood flow. These haemodynamic changes are believed to arise from actions of the anaesthetic agents on the autonomic nervous system, myocardial contractility, baroreceptor reflex function and peripheral vascular smooth muscle tone. ECs play a key role in regulating the contractile state of vascular smooth muscle, by releasing contracting (thromboxane and endothelin) or relaxing factors [nitric oxide (NO), prostacyclin and endothelium-derived hyperpolarizing factor (EDHF)]. The role of the endothelium in the haemodynamic effects of anaesthetics is highlighted by studies showing that these agents have different effects on isolated vessels with and without endothelium. In vivo and in vitro studies have made use of pharmacological inhibitors, and direct or indirect measurements of the release of endothelium-derived vasoactive factors, to show that volatile or i.v. anaesthetics alter endothelial production of NO, prostacyclin, EDHF, but also endothelin and possibly thromboxane. The alterations can be an increase or decrease of the release of vasoactive factor, are drug-specific, and vary depending on the dose of anaesthetic agent, the vascular bed studied, and whether the agent is applied alone or in combination with a vasoactive agonist. For example, propofol stimulates the release of NO by EC, enhances endothelial-dependent vasodilation, and prevents stress-induced impairment of endothelial production of NO. However, propofol has also been shown to attenuate acetylcholine-induced relaxation of pulmonary arterial rings, and this effect was, in part, NO-mediated. How can so many drugs differing in structure and mechanism of pharmacological action alter haemodynamic function? Endothelial caveolae could be a common target resulting in a variety of general anaesthetic-induced circulatory effects.

Caveolae are specialized subdomains of the plasma membrane found in most cell types, and particularly abundant in EC of continuous endothelium. Electron microscopy allows the visualization of caveolae as ~70 nm omega-shaped invaginations of the plasma membrane or as circularized single or clustered vesicles underneath the plasma membrane (Fig. 1). Caveolae serve signalling and trafficking functions, which are crucial to the physiology of EC and are believed to require caveolin-1, a major protein component of caveolae. A number of signalling molecules (lipids and proteins) have been localized to caveolae, in particular, molecules involved in crucial functions of ECs such as the regulation of haemostasis, vascular permeability, inflammation, blood vessel tone or angiogenesis.

Caveolin-1 plays a double role in signalling by (i) compartmentalizing signalling molecules and (ii) regulating their activity. Examples of proteins inhibited by interaction with caveolin-1 include, heterotrimeric G-protein α-subunits and endothelial NO synthase (NOS, type III). In contrast, in EC prostacyclin synthase has also been localized to caveolae where it interacts with caveolin-1, but the interaction does not seem to reduce its synthase activity.

Caveolin-1 is known to clinicians as a major regulator of endothelial NO production. The physiological importance of endothelial NOS interaction with caveolin-1 has been demonstrated in vivo by the inhibition of NO synthesis after intraperitoneal delivery of a peptide based on the
sequence of the scaffolding domain of caveolin-1 in mice. Interestingly, production of NO by ECs has been shown to be the target of volatile or i.v. anaesthetics. In addition, many studies suggest that anaesthetics could affect vasomotor tone by altering calcium entry in different cell types including ECs. Caveolae play a key role in calcium entry and Ca$^{2+}$-dependent signalling. A mechanism of cellular Ca$^{2+}$ entry activated when internal Ca$^{2+}$ stores are depleted and called store-operated Ca$^{2+}$ entry, or capacitative Ca$^{2+}$ entry (CCE), has been shown to be required for endothelial NOS activation. The signalling machinery involved in CCE is organized in caveolae into complexes which are functional in living ECs, and can locally stimulate NOS. Thus, calcium and NO signalling which are both altered by anaesthetics in EC are compartmentalized in caveolae.

Another function of the endothelium which is both dependent on caveolae and altered by anaesthetics is the regulation of endothelial permeability. Caveolae internalization and shuttling of macromolecules between the luminal side of the blood vessel and the sub-endothelial space (a process named transcytosis) is one of the first proposed functions of these organelles. The transcytosis of albumin, insulin, and low-density lipoproteins (LDL) by caveolae has been demonstrated. Isoflurane has been shown to increase the uptake and trans-endothelial transport of albumin in vitro and the phosphorylation of caveolin-1, which occurs when caveolae are endocytosed. This suggests that the increase in albumin transport could be a result of an increased internalization of caveolae, but awaits demonstration that isoflurane has less or no effect on transport of albumin in ECs devoid of caveolae. It should be noted that caveolae control vascular permeability by their ability to perform transcytosis, but also through the aptitude of caveolin-1 to regulate endothelial NO production, and by compartmentalizing specific receptors playing major functions in the control of vascular permeability.

Cholesterol is essential to caveolae integrity. In caveolae, and in non-caveolin-containing plasma membrane subdomains referred to as lipid rafts, cholesterol and sphingolipids are thought to be packed into a highly ordered structure distinct from the rest of the plasma membrane. Experimental acute or chronic depletion of cell cholesterol causes the loss of flask-shaped caveolae, the disassembly of the filamentous coat structure, and perturbations in signalling cascades originating in caveolae, presumably attributable to the mis-localization of caveolar signalling proteins. Alterations in membrane cholesterol can be achieved using cholesterol binding drugs, such as methyl-β-cycloexdrin, filipin or nystatin, and oxidized LDL, or the bacterial enzyme cholesterol oxidase. It should be noted that several general anaesthetics have membrane-perturbing properties, some of which are cholesterol-mediated. General anaesthetics could therefore perturb the lipid composition or the ordered structure of caveolae, altering protein–protein interactions or the proximity between signalling proteins, with potential consequences on downstream signalling. Even though a physical effect of anaesthetics on the lipids of the plasma membrane does not appear today to be the mechanism for anaesthesia (which rather involves direct interaction of the anaesthetic with proteins or perturbation of the protein–lipid interface), volatile anaesthetics have been shown to affect membranes: they increase the internal fluidity of phospholipids–cholesterol bilayers, induce a cholesterol-dependent change in the surface potential of the membrane, a release of surface-bound water resulting in decreased surface viscosity, and a decrease of microviscosity in biological membranes. Surprisingly, propofol is also able to fluidize membranes with a much greater potency than anticipated from its lipophilicity.

Because caveolae are specialized plasma membrane subdomains with a distinct lipid composition which compartmentalize the signalling proteins essential to the endothelial functions that are altered by anaesthetics, it is tempting to hypothesize that caveolae are a common target for the endothelial-mediated circulatory effects of anaesthesia. This represents a new area of research extremely attractive to anaesthetists. Halothane has been shown already to partition into subdomains of the plasma membrane similar to caveolae, and isolated biochemically based on their lipid composition (lipid rafts), where it was shown to bind numerous proteins. This is the only evidence published so far that anaesthetics might alter lipid rafts, caveolae, or both.

Caveolae and caveolin-1 exert dual roles on signalling and trafficking functions. On the one hand, the caveolae structure is necessary to the existence of signalling platforms where proximity between receptors and effectors is crucial. On the other hand, caveolin-1 exerts an inhibitory action on several of the signalling proteins bound to its scaffolding domain. Disrupting this interaction and disrupting caveolae can have opposite effects on a specific signalling pathway, as depicted in Figure 2. Caveolae as a common target for different anaesthetics could explain why the effects described in the literature can be contradictory depending on the dose of anaesthetic (e.g. at a low dose, the inhibitory effect of caveolin-1 is decreased, at higher doses, the whole signalling pathway is disrupted) and depending on whether or not they are assessed in experimental conditions which include agonists (i.e. perturbing caveolae can relieve the tonic inhibition by caveolin-1 but impair agonist-induced signalling).
Caveolae could well be an important link reconciling discrepant results on the circulatory effects of general anaesthetics. Both this organelle and its defining protein are involved in trafficking and in the compartmentalization and regulation of signalling proteins. Whether the structure and function of caveolae are affected by cell exposure to caveolae plasma membrane, or to the cytoplasm. (c) When stimulated by its agonist A, a receptor R translocates into caveolae where it encounters and interacts with effector E, resulting in signal S. In the presence of an anaesthetic, caveolae structure is perturbed, the activated receptor cannot meet and activate the effector, which prevents the generation of the signal S.

Fig 2 Schematic depicting the hypothetical effects of anaesthetics on EC caveolae. (a) Caveolin-1 exerts a tonic inhibition on a signalling protein (E) in caveolae. In the presence of anaesthetic (thunder shape), the interaction is disrupted, the inhibition released and the signal (S) increased from E, which can either stay in caveolae, move to non caveolae plasma membrane, or to the cytoplasm. (b) When activated by its agonist A, a receptor R signals to an effector E resulting in signal S. In the presence of an anaesthetic, the proximity of the receptor and the effector within caveolae is abolished, resulting in a reduction or absence of downstream signalling. (c) When stimulated by its agonist A, a receptor R translocates into caveolae where it encounters and interacts with effector E, resulting in signal S. In the presence of an anaesthetic, caveolae structure is perturbed, the activated receptor cannot meet and activate the effector, which prevents the generation of the signal S.

cardiovascular system will no doubt be the object of the attention of anaesthetists in the next few years. Experiments could make use of caveolin-1 gene-disrupted mice, whose phenotype includes aberrant NO and calcium signalling in the cardiovascular system, and NO-dependent microvascular hyper-permeability.

M.-O. Parat

Cleveland, OH, USA
E-mail: paratm@ccf.org

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